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Yours sincerely

E. J. Butler

UNIV. OF MICH.

SIR EDWIN JOHN BUTLER, C.M.G., C.I.E., D.Sc., LL.D., F.R.S.
1874–1943

H. T. Güssow

Sir Edwin, notwithstanding a cordial relationship with numerous mycologists and plant pathologists on this Continent, never became a member of The American Phytopathological Society. However, many of us whose privilege it has been to know this eminent and lovable man, who after long and faithful service to our profession has passed from this life into one of everlasting peace and glory, feel that a place of honor may be fitly accorded him in our journal. Sir Edwin passed away on April 4th this year, after many years of loyal service in India, the climate of which undermined his health, as has so often been the case with many a stronger man. He had been very ill for the last two years—his heart grew steadily weaker—influenza set in, and the strain was too great. . . .

Born in County Clare, Ireland—educated at Queen's College, Cork—senior scholar in 1896—he was graduated in medicine (M.B. Hons.) at the Royal University of Ireland, but, like so many of his British colleagues of similar training, he favored Botany as his life's calling. During a travelling scholarship (1899–1900) in Paris, Freiburg—of which he often spoke with pride and pleasure—the Antibes, and Kew, he interested himself in the study of water moulds and related fungi.

In 1901 he received his appointment as cryptogamic botanist to the Government of India, and at once threw his heart and soul into the mycological problems of the great Empire he served with such distinction. In 1905 he went to Pusa as Imperial Mycologist at the Imperial Agricultural Research Institute, where his first task was to train workers in the methods of botanical and mycological research. He established for himself the reputation of a capable and inspiring teacher, commanding the respect and gaining the affection of a large staff who soon became devoted to this sincere, patient, and kindly man.

His earlier interests culminated in his still classical Memoir: "An account of the genus Pythium and some Chytridiales" (1907). With H. and P. Sydow he commenced a series of special articles (Fungi Indiae orientalis 1906–1916)—which laid the foundation to "The Fungi of India" published jointly with his good friend and collaborator, G. R. Bisby, formerly of the University of Manitoba but soon joining Butler's staff at the Imperial Mycological Institute at Kew. Among his numerous contributions to our science, his "Fungi and Disease in Plants" (Calcutta, 1918), perhaps more than any other, established his international reputation. Prior to his lamented death, Butler engaged in rewriting the first chapter for a new book he had long in mind, and in which he wished to summarize a life's long experiences.

The selection of so experienced and distinguished a man as first Director of the Imperial Mycological Bureau (now Institute) in 1921 was indeed a

wise and happy choice, and one that soon made the Institute, under his direction, one of the world's most famous. In 1922 this indefatigable worker, assisted by a devoted staff, brought out the first number of the "Review of Applied Mycology," which, as I have been told often by workers throughout the universe, "is the first periodical consulted when one takes up a new problem." The "Review" lives and surpasses in its scope and its conception similar publications in any language. In 1924 Butler inaugurated the first "Imperial Mycological Conference" between workers of the British Empire. During these, I became intimately befriended, enjoying often the great privilege of being his house guest and that of his charming lady. In 1926 Butler was elected a Fellow of the Royal Society of London (in 1934 to the council of the Society); in 1932 he was awarded the C.M.G.; in 1938 the old University of Aberdeen honored him with the degree of LL.D., and in 1939 he received his knighthood in recognition of his meritorious and outstanding services to his Empire. Finally, in 1935, he could no longer—as he told me—"in self respect" resist the urgent demands made upon him by the Agricultural Research Council of Great Britain and became its secretary. The destiny of the Institute, which he so successfully guided, then passed on to his trusted Assistant Director, S. F. Ashby, B.Sc., succeeded on the latter's retirement by Dr. S. P. Wiltshire, who has before him the task of carrying on in the spirit of its founder.

"Exegi monumentum . . . quod non . . . annorum et fuga temporum possit diruere."

MULTIPLICATION OF VIRUSES IN THE DODDER, CUSCUTA CAMPESTRIS

A. S. COSTA[1, 2]

(Accepted for publication September 16, 1943)

INTRODUCTION

Bennett (1) was the first to report that species of *Cuscuta* could be used to transmit certain plant viruses. Working with two species of this genus, *Cuscuta subinclusa* Dur. and Hilg. and *C. californica* Choisy, he was able to transmit cucumber-mosaic virus from tobacco to tobacco and also to *Nicotiana glauca* Grah. Moreover, he was able to transmit curly top of sugar beet by means of *C. subinclusa*.

The transmission of viruses through dodder was discovered independently by Johnson (3, 4), who succeeded in transmitting aster-yellows, tomato bushy-stunt, sugar beet curly-top, tobacco-mosaic, and white clover-mosaic viruses by means of the parasitic activities of *Cuscuta campestris* Yuncker.

Kunkel (5) reported transmission of cranberry false-blossom virus to several species of plants through the same dodder used by Johnson. He was the first to emphasize (6) that the great importance of this new method of transmitting plant viruses lies in the fact that many viruses heretofore obtained in a single or in few related species could by this means be transmitted to new hosts in which they could be studied to better advantage.

The role played by dodder in the transmission of viruses is not yet fully understood. Bennett (1) reported that *Cuscuta subinclusa* becomes infected with cucumber-mosaic virus and retains the virus indefinitely on immune plants. Johnson (4) pointed out that neither tobacco-mosaic virus, which was transmitted, nor tobacco-ring-spot virus, which was not transmitted, multiplies in dodder. No reference was made to the other viruses with which he worked.

The present paper reports results of a study of the role played by the dodder, *Cuscuta campestris,* in the transmission of 4 viruses: ordinary tobacco-mosaic virus (*Marmor tabaci* H. var. *vulgare* H.), aucuba-mosaic virus (*M. tabaci* H. var. *aucuba* H.), cucumber-mosaic virus (*M. cucumeris* H. var. *vulgare* H.), and cranberry false-blossom virus (*Chlorogenus vaccinii* H.).

MATERIAL AND TECHNIQUE

The experiments herein reported were conducted in one of the greenhouses of The Rockefeller Institute for Medical Research, Princeton, New Jersey, from January to June, 1943.

[1] Fellow of The Rockefeller Foundation.

[2] The writer is greatly indebted to Dr. L. O. Kunkel, Dr. F. O. Holmes, and other members of The Rockefeller Institute for Medical Research, Princeton, N. J., for advice and criticism offered during the investigation reported in this paper and during preparation of the manuscript.

Source of Viruses and Dodder

The viruses used in these experiments were obtained from the collection carried at this Institute. Susceptible plants were first infected mechanically or by grafting with the virus desired. After the inoculated plants showed systemic symptoms, healthy dodder stems were established on the diseased plants and the dodder growth developed on them constituted the source of viruliferous dodder. A constant supply of healthy dodder was kept on tomato or tobacco plants. All experiments were made with *Cuscuta campestris*.

Transfer of Vector

In all cases dodder stems 4–8 inches long were used in making transmissions. This was found more desirable than connecting the test plants to dodder that was still attached to diseased plants. It not only diminished the chances of contamination but also facilitated the handling of individual plants. The dodder stems were always arranged around suitable parts of the host plants, mostly around the younger parts of the stem. The dodder growth was in general left undisturbed on the test plants for about 15 days; then it was pulled off. However, care was taken to prevent connections between adjoining plants, the advancing tips of the dodder stems sometimes being pinched off before the 15 days had elapsed.

Test Plants

Plants of *Lycopersicon esculentum* Mill. var. Bonny Best were used exclusively in the experiments with cranberry false-blossom virus both as sources of inoculum and as test plants. The alfalfa plant, *Medicago sativa* L., served as a plant immune from cranberry false-blossom virus.[3] Tomato and tobacco plants were used in studying tobacco-mosaic and aucuba-mosaic viruses. Crimson clover (*Trifolium incarnatum* L.) and alfalfa were used as plants immune from these viruses. For cucumber-mosaic virus, tobacco and *Nicotiana glutinosa* L. served both as sources of virus and as test plants. In the beginning, soybean, *Glycine max* Merr., was used as a plant immune from the virus (7), but the dodder grew poorly on it. Later trials were, therefore, made with the red clover, *Trifolium pratense* L., also immune from this virus (7).

In most cases dodder was placed on the host plants when they were very young. Not only was it easier to establish the dodder on young plants, but the incubation periods of the diseases were shorter than in old plants.

The development of symptoms was used as a criterion of transmission in the case of cranberry false-blossom virus. With cucumber-mosaic, ordinary tobacco-mosaic, and aucuba-mosaic viruses, judgment as to transmission was based not only on the development of symptoms but also on results of subinoculations to tobacco, *Nicotiana glutinosa*, *N. sylvestris* Speg. and Comes, or *Vigna sinensis* (L.) Endl. var. Black. The host plants that

[3] Kunkel, L. O. Unpublished work.

showed no symptoms were tested by mechanical inoculation to one or another of these species shortly before being discarded. The immune plants serving as hosts for the dodder also were tested for the virus concerned by subinoculation to one of these susceptible species.

In experiments with cranberry false-blossom virus, the tomato test plants were kept for observation until 90 days after the dodder stems had been established on them. In the cases of tobacco mosaic and aucuba mosaic, the test plants were kept only 40 days, being then tested and discarded. With cucumber-mosaic virus, the test plants were discarded 30 days after they had been parasitized by dodder.

Controls

Healthy dodder stems were placed on the control plants in the case of cranberry false-blossom virus. In the experiments made with viruses mechanically transmissible, control plants were parasitized with viruliferous dodder stems in the same manner as the test plants. A short time after this had been done, the viruliferous dodder was removed and a healthy dodder stem substituted. It was thought that this procedure would afford a check against the possibility of mechanical contamination when placing the dodder on the test plants.

Other details regarding technique will be given with data on the experiments concerned.

EXPERIMENTAL

Negative Evidence on the Multiplication of Ordinary Tobacco-Mosaic and Aucuba-Mosaic Viruses in Dodder

In a study of the multiplication of ordinary tobacco-mosaic and aucuba-mosaic viruses in dodder, an attempt was made first to measure the virus in dodder stems quantitatively by mechanical inoculation. Soon it was learned that the dodder juice had an inhibitory effect on both viruses and that both occurred only at a very low concentration in dodder.

Inhibitory Action of Dodder Juice on Ordinary Tobacco-Mosaic and Aucuba-Mosaic Viruses. Preliminary attempts to recover ordinary tobacco-mosaic virus from dodder stems grown on diseased plants showed that very little virus could be recovered. Three experiments were made. In each, 30 leaves of *Nicotiana glutinosa* and 10 Turkish tobacco plants were rubbed with dodder juice from stems grown on diseased plants. Carborundum was added to the inoculum. A total of 37, 15, and 20 lesions was obtained on *N. glutinosa* and a total of 9, 5, and 4 infected tobacco plants, respectively. Without the addition of carborundum it was difficult to get a single lesion on *N. glutinosa*. Similar results were obtained with aucuba-mosaic virus.

In table 1 results are given of experiments on the inhibitory action of dodder juice on ordinary tobacco-mosaic virus as measured on *Nicotiana glutinosa*. The virus solutions were prepared at 3 concentrations (10^{-1}, 10^{-2}, and 10^{-3}) in water or 0.1 M neutral phosphate buffer. Equal parts

TABLE 1.—*Inhibitory action of dodder juice on tobacco-mosaic virus as measured by the number of local lesions secured on Nicotiana glutinosa. Concentration of dodder juice 1 : 2*

Experiment No.	Dilution, treatment, and average number of lesions[a]					
	$\frac{1}{2} \times 10^{-1}$		$\frac{1}{2} \times 10^{-2}$		$\frac{1}{2} \times 10^{-3}$	
	Dodder juice	Control	Dodder juice	Control	Dodder juice	Control
1[b]	9.9	52.8	2.0	15.2	0.1	7.3
2[b]	15.9	84.6	4.1	43.5	1.0	10.1
3[b]	7.3	23.9	2.6	20.6	0.6	6.5
4[c]	2.1	29.3	0.4	9.8	0.3	4.8
5[c]	4.8	43.5	0.5	22.3	0.1	6.4
Average	8.0	46.8	1.9	22.3	0.4	7.0

[a] Average number of lesions per half-leaf based on 10 half-leaves.
[b] Diluent distilled water.
[c] Diluent 0.1 M phosphate buffer at pH 7.

of virus solutions and normal dodder juice were then mixed shortly before inoculation. Controls were brought to the same dilution by addition of water or buffer, as required. The results show that at the 3 dilutions tested, viz., $\frac{1}{2} \times 10^{-1}$, $\frac{1}{2} \times 10^{-2}$, and $\frac{1}{2} \times 10^{-3}$, the same amount of dodder juice reduced the infectivity of the inoculum by 82.9, 91.5, and 94.3 per cent, respectively. The results suggest that the action of the inhibitor is, in part, on the virus itself (2, 8).

The low concentration of the virus in dodder grown on diseased plants and the inhibitory action of the dodder juice may perhaps explain why Bennett (1) could not recover the virus by mechanical inoculation from *Cuscuta subinclusa* grown on diseased plants.

Experiments on the Transmission of Ordinary Tobacco-Mosaic and Aucuba-Mosaic Viruses by Dodder Taken Directly from Diseased Plants and

TABLE 2.—*Transmission of tobacco-mosaic and aucuba-mosaic viruses by the dodder, Cuscuta campestris, from tomato to tomato*

Virus of	Test plant	Nature of dodder stems used to parasitize the test plants		
		Dodder from diseased plants	Dodder originally grown on diseased plants and then transferred through a series of immune plants	
			After 1st transfer	After 2nd transfer
Tobacco mosaic	*Lycopersicon esculentum* var. Bonny Best	5/31[a]	0/48	0/10
–	Control	0/10	0/20	0/5
Aucuba mosaic	*Lycopersicon esculentum* var. Bonny Best	2/28	0/23	0/19
–	Control	0/10	0/10	0/10

[a] The numerator indicates the number of infected plants and the denominator the number of plants that were parasitized with the viruliferous dodder.

after Transfers Through Immune Plants. Owing to the difficulty of measuring ordinary tobacco-mosaic and aucuba-mosaic viruses in dodder stems by quantitative methods based on local lesions, it was decided to investigate the behavior of these viruses in dodder by making successive transfers of viruliferous dodder stems on immune plants. After each transfer, the dodder growth was tested for virus by mechanical inoculation and by transfer to susceptible host plants. It was thought that these transfers would provide an increasing dilution of the viruses unless they could multiply in dodder.

In tables 2 and 3 are presented the results of transmission of ordinary tobacco-mosaic and aucuba-mosaic viruses through dodder when taken directly from diseased plants and after one and two transfers on immune plants. It will be seen that in a few cases transmission was obtained through dodder taken directly from diseased plants, but that in no case was

TABLE 3.—*Transmission of tobacco-mosaic and aucuba-mosaic viruses by the dodder, Cuscuta campestris, to tobacco plants*

Virus of	Test plant	Dodder stems grown on diseased plants		Control
		From tomato plants	From tobacco plants	
Tobacco mosaic	Turkish tobacco	1/4[a]	1/4	0/4
Aucuba mosaic	" "	1/18	.....	0/9

[a] See footnote, table 2.

transmission obtained after the first or second transfer. The mechanical inoculations confirmed this point. No lesions were obtained on *Nicotiana glutinosa* inoculated with dodder extract taken after the transfers.

These results confirm the view of Johnson (4) that the ordinary tobacco-mosaic virus does not multiply in dodder and show that this is also true for the aucuba-mosaic strain.

Multiplication of Cucumber-Mosaic Virus in the Dodder, Cuscuta campestris

The results obtained with cucumber-mosaic virus in dodder, *Cuscuta campestris,* confirmed the findings of Bennett (1) that cucumber-mosaic virus infects and multiplies in dodder.

Transmission of Cucumber-Mosaic Virus by Viruliferous Dodder Grown on Immune Plants. Dodder stems grown on diseased plants transmitted cucumber-mosaic virus to almost all susceptible plants on which they were placed and established. In one experiment, 7 tobacco plants of 9 that had been parasitized with dodder from diseased plants became affected and 7 of 8 *Nicotiana glutinosa* plants. Four tobacco plants and 7 *N. glutinosa* plants used as controls remained healthy. In another experiment, 16 of 20 Turkish tobacco plants were infected and none of 10 controls. In a third experi-

TABLE 4.—*Transmission of cucumber mosaic virus through dodder taken directly from diseased plants and after transfers on immune plants*

Dodder tested by	Test plant	Nature of the dodder stems				
		From diseased plants	Grown on diseased plants and then transferred on immune plants after			
			1st transfer	2nd transfer	3rd transfer	4th transfer
Parasitizing the test plants	*Nicotiana tabacum* Turkish	4/6[a]	3/4	4/4	4/4	.....
Control	"	0/4	0/4	0/4	0/4	.....
By mechanical inoculation	*N. tabacum*	5/5[b]	5/5	5/5	5/5	5/5
Control	"	0/5	0/5	0/5	0/5	0/5
By mechanical inoculation	*N. glutinosa*	5/5	5/5	5/5	.....	5/5
Control	"	0/5	0/5	0/5	.....	0/5

[a] See footnote, table 2.

[b] Denominator gives the number of plants inoculated and the numerator the number of plants infected.

ment, all of 7 Turkish tobacco plants were infected and none of 5 controls. The incubation period in these experiments varied from 9 to 20 days.

In table 4 are presented some results comparing the transmission of cucumber-mosaic virus through dodder taken directly from the diseased plants and after transfers on immune plants. From the table it can be seen that, even after the 4th transfer, virus was present in dodder at approximately the same concentration as in dodder grown on diseased plants. Calculations showed that the 4th transfer would correspond to a dilution of about 1 : 5,000,000 of the initial virus concentration in the dodder. This is beyond the dilution end point of the virus. During the transfers the virus was retained in dodder for 4 months.

Mechanical Inoculation of Dodder Stems with Cucumber-Mosaic Virus. Additional evidence that cucumber-mosaic virus infects dodder and multi-

TABLE 5.—*Mechanical inoculation of the dodder, Cuscuta campestris, with cucumber-mosaic virus*

Experiment No.	Test plant and number of dodder stems out of the total inoculated that transmitted the virus			
	Nicotiana tabacum		*Nicotiana glutinosa*	
	Dodder inoculated mechanically	Control	Dodder inoculated mechanically	Control
1	4/8[a]	0/4	3/8	0/4
2	1/8	0/4	0/8	0/4
3	2/14	0/8	.....	.....
Total	7/30	0/16	3/16	0/8

[a] The denominator indicates the number of dodder stems that were inoculated and the numerator the number that transmitted the virus to the test plants.

plies in it was obtained by mechanical inoculation of dodder stems. This was done by rubbing vigorous dodder stems 4–8 inches in length, along $\frac{2}{3}$ of the basal parts, with a small cotton swab dipped in the inoculum to which carborundum had been added. Undiluted juice or juice diluted 1:5 with 0.1 M neutral phosphate buffer was used. The rubbing was not done near the tips of the dodder stems because it had been found that, when the tips were rubbed, it was difficult to obtain establishment of the dodder on host plants. After inoculation the stems were rinsed in water and placed on susceptible plants and also on immune plants. In table 5 are presented the results obtained when the inoculated dodder was placed on Turkish tobacco or on *Nicotiana glutinosa*. Table 6 gives the results when the inoculated dodder was placed on red clover plants and then tested for virus at different intervals of time. In a few cases the inoculated dodder stems transmitted the virus to the susceptible plants on which they were placed or virus

TABLE 6.—*Number of dodder stems inoculated mechanically with the cucumber-mosaic virus and from which the virus was recovered after they had been established on red clover, Trifolium pratense. Recovery by mechanical inoculation to tobacco and Nicotiana glutinosa plants*

Experiment No.	Extract from inoculated dodder grown on immune plants. Tested after		
	1 day	7 days	15 days
1	0/10[a]	.....	4/10
2	0/10	2/10	2/20
3	0/10	0/20	0/20
Total	0/30	2/30	6/50

[a] The denominator indicates the number of inoculated dodder stems that were tested and the numerator the number that contained the virus as tested by mechanical inoculation.

was recovered from them after they had been established on immune plants. The evidence indicates that the virus infected the dodder and multiplied in it. When inoculated dodder was placed on susceptible plants, it could be conceived that the virus mechanically introduced into the dodder might pass to the test plant and cause infection. However, this would not be possible when the dodder was placed on red clover plants that were immune from the virus. The clover plants were tested for virus with negative results.

Local Lesions Caused by Viruliferous Dodder at the Point of Attachment. In preliminary experiments, dodder stems containing cucumber-mosaic virus established on *Vicia faba* L. plants caused the appearance of black elongated necrotic lesions at the point of attachment. Control plants on which healthy dodder had been established showed no such lesions. In another experiment, local lesions were observed in 9 of 10 plants on which viruliferous dodder stems were placed and in none of 3 controls. The local lesions were visible from 5 to 7 days after the dodder had been placed on the test plants. In a third experiment, necrosis near the point of attachment

began to show on the 4th day after the dodder had been placed on the plants. Leaves of *V. faba*, when inoculated by rubbing with the cucumber-mosaic virus, show necrotic local lesions in about 2 or 3 days. The lesions near the point of attachment of the dodder may appear in 4 days. This is a very short time if we consider that it takes 1 or 2 days for the dodder to make a haustorial connection with the host plant. It also shows that infection occurs almost immediately after organic union between dodder and host plant takes place and that virus is present in the cells involved in the process of haustoria formation.

Local lesions near the point of attachment also were obtained when viruliferous dodder stems were placed on *Vigna sinensis*. The lesions were reddish but not very conspicuous.

Recovery of Cucumber-Mosaic Virus from Dodder Stems and Inhibitory Effect of Dodder Juice. Preliminary attempts to recover the virus from

TABLE 7.—*Inhibitory effect of dodder juice on the cucumber-mosaic virus as measured by the number of local lesions secured on Vigna sinensis var. Black. Virus diluted to 1:5. Diluent 0.1 M neutral phosphate buffer*

Experiment No.	Concentration of dodder juice and average number of lesions[a]					
	0	3:5	2:5	1:5	1:25	1:125
1	15.5	0	0	0	—	—
2	37.0	0	0	0	—	—
3	15.6	—	—	—	0.4	2.1
4	39.8	—	—	—	0.2	3.6

[a] Average number of lesions per leaf on 30 leaves.

dodder stems grown on diseased plants showed that, although some virus was present, it was difficult to demonstrate this by inoculation to *Vigna sinensis*, in which the virus produces local lesions. Virus could with some difficulty be recovered by rubbing the dodder juice onto tobacco plants. Virus could also be demonstrated in dodder by placing it on host plants that would become infected.

Some trials were made by mixing cucumber-mosaic virus inoculum with different amounts of dodder juice. In one trial the virus concentration was kept constant at 1:5 and the dodder juice was tried at 3 concentrations: 1:5, 2:5, and 3:5. Each concentration of inhibitor was inoculated on 30 leaves of *Vigna sinensis*, the opposite leaves being inoculated with a control preparation containing no dodder juice. Carborundum was always used for inoculation. The results presented in table 7 show that at 1:5 the dodder juice completely inhibited the infectivity of the inoculum as tested on *V. sinensis*. A second experiment gave identical results. A 3rd and 4th experiment were then made by adding 1 cc. of dodder juice at various concentrations to a mixture of 1 cc. cucumber-mosaic virus extract + 3 cc. of 0.1 M phosphate buffer at pH 7. Two concentrations of dodder juice were used, 1:5 and 1:25. The final concentration of virus after mixture

with dodder juice was 1:5 and of dodder juice 1:25, 1:125, and control. The results show that, even at 1:125, the dodder juice had a pronounced inhibitory action. The data obtained do not permit a statement as to whether the inhibitory action is on the virus or on the host plant.

Symptoms Exhibited by Dodder Infected with Cucumber-Mosaic Virus. Apparently, dodder stems infected with cucumber-mosaic virus show some symptoms of disease. This was observed as an almost constant feature in the experiments, and, whereas no certainty exists as to its nature, it seems worthy of being reported.

The symptoms consist of a distorted type of growth. This is illustrated in figure 1. The degree of distortion may vary, but it can always be per-

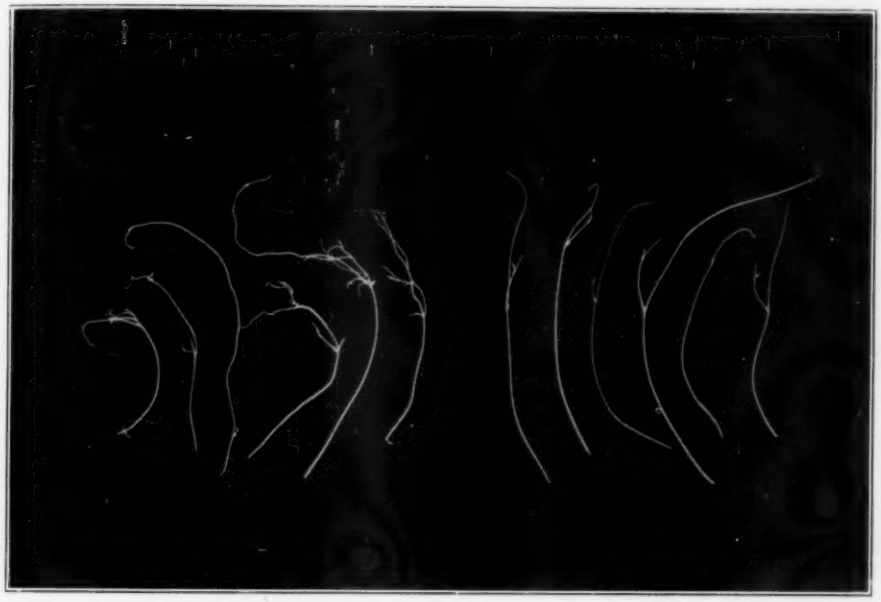

FIG. 1. The distorted type of growth exhibited by dodder stems infected with cucumber-mosaic virus. Healthy dodder stems at the right. (Photograph by J. A. Carlile.)

ceived. The distorted type of growth could, in most instances, be noticed after the viruliferous dodder had been transferred on a series of immune plants. In addition, the growth of viruliferous stems was apparently slower than normal. It was also found much more difficult to establish the dodder on a plant affected by cucumber mosaic than on plants affected by tobacco mosaic or on healthy controls.

The distortion above described may occasionally be brought about by adverse conditions. There may remain some doubt, therefore, whether the observed symptoms are really the result of the virus. In no case, however, were these symptoms found associated with tobacco-mosaic virus or cranberry false-blossom virus.

Multiplication of Cranberry False-Blossom Virus in the Dodder, Cuscuta campestris

The multiplication of cranberry false-blossom virus in dodder was studied by successive transfers of viruliferous dodder on a series of immune alfalfa plants. By this means an increasing dilution of the virus present in dodder took place. Six transfers were made in one experiment and 5 transfers in each of 4 other experiments. The calculations showed that each transfer would correspond to at least a tenfold dilution. After each transfer, samples of dodder stems were placed on tomato test plants and permitted to become established in the manner already described. Table 8 records the results obtained.

TABLE 8—*Transmission of cranberry false-blossom virus by the dodder, Cuscuta campestris. Test plant, tomato. Immune plant, alfalfa*

Experiment	Nature of the dodder stems used to parasitize the test plants						
	From diseased plant	Grown on diseased plants and then transferred on immune plants after					
		1st transfer	2nd transfer	3rd transfer	4th transfer	5th transfer	6th transfer
1	18/23[a]	27/31	10/18	9/19	7/21	0/24	5/38
2	15/20	9/14	11/15	19/27	20/26	9/23	.....
3	8/10	12/15	4/15	5/9	3/11	12/27	.....
4	13/20	11/16	10/18	11/29	15/22	3/16	.....
5	15/20	13/16	8/16	8/16	9/25	4/19	.....
Total	69/93	72/92	43/82	52/100	52/105	27/109	5/38
Control	0/25	0/25	0/23	0/20	0/22	0/25	0/5

[a] See footnote, table 2.

As can be seen from the table, there was a decrease in the percentage of infected plants after the transfers, but this decrease was not marked and apparently was not due to dilution. Two possible explanations may account for this. First, it may be said that the decrease was due to environmental conditions. The first transfers were performed in winter when the greenhouse temperature was kept around 75° F. The last transfers, on the other hand, were made in summer when the greenhouse temperature sometimes goes very high. Kunkel (5) has already shown that cranberry false-blossom virus is sensitive to heat. The second explanation is that when the dodder stem is taken from a diseased plant, the virus titer may be higher than after transfer to an immune plant. This is easy to understand because, in addition to the virus infecting the dodder, some virus might be absorbed from the diseased plant along with food.

It is interesting to note that, in addition to withstanding a dilution of at least 10^{-6}, the virus was retained in dodder for about 100 days. Considering these facts, it seems reasonable to conclude that cranberry false-blossom virus multiplies in dodder.

DISCUSSION

Tobacco-mosaic and aucuba-mosaic viruses, although highly infectious, are transmitted through dodder taken from diseased plants in only a small percentage of cases and not through dodder from diseased plants after 1 or 2 transfers on immune plants. This constitutes evidence that these viruses do not multiply in dodder. The period of incubation (longer than in mechanical transmission) also confirms this point and suggests that transmission is dependent upon the movement of materials through the dodder rather than upon close contact of infected dodder tissue with the host plant.

Cucumber-mosaic virus shows a different behavior in its transmission through dodder. The virus is easily transmitted through viruliferous dodder. The incubation period is relatively short, the majority of plants showing symptoms from 9 to 12 days after the viruliferous dodder stems are placed on them. Moreover, the viruliferous dodder retained the virus after 4 successive transfers on immune hosts made during a period of 4 months, without an apparent decrease in virus concentration. Experiments with *Vicia faba*, on which the viruliferous dodder causes local lesions near the point of attachment, showed that passage of virus from the dodder to the host plant occurs probably at the time of haustoria formation. This suggests that the virus becomes systemic in dodder and that the cells involved in haustoria formation contain it. Dodder stems could also be inoculated mechanically with cucumber-mosaic virus. This, too, is evidence that the virus is able to infect the dodder and multiply in it. The observation that certain symptoms of disease persisted in dodder stems that had been grown on diseased plants and transferred to immune plants also supports the view that this virus infects the dodder. Additional evidence as to the infection of dodder stems by cucumber-mosaic virus could perhaps be obtained by protection tests. Infection of dodder with cucumber-mosaic virus by aphids would also furnish additional evidence on multiplication.

The cranberry false-blossom virus could be recovered from viruliferous dodder after 6 successive transfers on immune plants made during a period of 4 months. This corresponds to a dilution of at least 10^{-6}. The evidence is highly suggestive that the virus multiplies in the dodder. The number of plants infected by viruliferous dodder is also relatively high.

SUMMARY

The ordinary tobacco-mosaic and aucuba-mosaic viruses were transmitted through the dodder, *Cuscuta campestris*, in a few cases when the dodder was taken directly from diseased plants. The incubation period varied from 12 to 20 days. No case of transmission was obtained after dodder from diseased plants had been transferred once or twice through alfalfa or crimson clover plants immune from the virus.

Dodder juice has an inhibitory effect on ordinary tobacco-mosaic and aucuba-mosaic viruses as measured by the number of local lesions secured on *Nicotiana glutinosa*. Apparently the action is, in part, on the virus.

High percentage of transmission through dodder was obtained with cucumber-mosaic virus. Viruliferous dodder stems, grown after 4 successive transfers through red clover immune from the virus, showed no decrease in virus content. These 4 transfers would correspond to a dilution beyond the dilution end point of the virus. The incubation period varied from 9 to 20 days, most plants showing symptoms in from 9 to 12 days.

Local lesions near the point of attachment were obtained by placing dodder stems containing cucumber-mosaic virus on *Vicia faba*. The lesions appeared in 4 to 5 days.

Dodder juice also had an inhibitory effect on cucumber-mosaic virus as measured on *Vigna sinensis* var. Black.

Dodder stems were successfully inoculated by rubbing with cucumber-mosaic virus. This was shown by placing the dodder on test plants or by establishing it on immune plants and then recovering the virus by inoculation.

Dodder stems infected by cucumber-mosaic virus showed in most cases a distorted type of growth. The degree of distortion varied, but it could always be perceived.

Cranberry false-blossom virus was retained in viruliferous dodder after 6 successive transfers on alfalfa, which is immune from this virus.

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ROOT INFECTION OF CROP PLANTS AND WEEDS BY TOBACCO LEAF-SPOT BACTERIA[1]

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Angular leaf spot and wildfire, caused by *Bacterium angulatum* F. and M. and *Bact. tabacum* W. and F., respectively, have caused loss in plant beds and in the field in Kentucky for many years. In burley plant beds angular leaf spot is almost certain to develop, when the plants are about ¼ grown, during a wet, cool period. It is rarely conspicuous in dark tobacco beds. During exceptionally wet springs, wildfire may break out in areas where it had not been observed before. For example, in the spring of 1943 wildfire could be found in nearly half of the untreated beds in Central Kentucky in areas where it was almost unknown before. No satisfactory explanation has been given for outbreaks of either disease in plant beds until recently. The explanations that the bacteria are seed-borne; that they are blown into beds on bits of infected tobacco trash; or that other plants that have become water-soaked and infected, harbor the bacteria for months, and then act as a source of infection, have not been adequate to explain general infection of tobacco beds following a wet period.

Another feature of the diseases that has needed explanation is that both diseases can be completely controlled in plant beds, as they are handled in Kentucky, if 2 applications of 3–4–50 Bordeaux are made to the surface of the soil when the plants are small. The only purpose of the second application appears to be to make certain that spots that may have been missed the first time are treated. The treatments are not designed to cover the leaves because, at the time of application, leaves that would later be susceptible have not yet developed. The purpose has been to coat thoroughly the surface of the soil with Bordeaux.

Recently, in Science,[2] we reported that both *Bacterium angulatum* and *Bact. tabacum* live on the roots of various crop plants and can be perpetuated in the field from one season to another by this means. The present paper presents evidence on this phase of the problem and its relation to outbreaks of the diseases in the plant bed and the field.

CARRY-OVER IN SOIL

Clinton and McCormick[3] demonstrated that collections of soil made in March and April, in a garden plot where wildfire-affected tobacco had grown the year before and allowed to rot in place, were capable of causing infection on pricked tobacco leaves in the greenhouse. Later studies by others did not

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Valleau, W. D., E. M. Johnson, and S. Diachun. Association of tobacco leaf-spot bacteria with roots of crop plants. Science 96: 2485, p. 164. 1942.

[3] Clinton, George P., and Florence A. McCormick. Wildfire of tobacco in Connecticut. Conn. Agr. Exp. Stat. Bull. 239. 1922.

confirm these results, and the tendency was toward not accepting the possibility of overwintering in moist soil. Wolf,[4] in 1922, found that some beds in which wildfire was present one year, if used the second year, sometimes developed wildfire when clean seed and new cotton were used. He believed this was evidence in favor of overwintering in soil. An abundance of the same kind of evidence in favor of general soil infestation can be obtained each year in areas where plant beds are placed in a new site and clean cotton is used. Wildfire and angular leaf spot are as likely to develop in these new sites as in old ones.

Positive proof of overwintering of *Bacterium angulatum* in soil was presented in abstract in 1942 by the present writers.[5] Using an improved technique for isolation of pathogenic bacteria from the soil, which consisted in watersoaking a leaf[6] and then pouring the soil to be tested, thoroughly stirred in an excess of water, over the undersurface, it was possible to show that the bacteria overwintered in considerable numbers in field soils in which crops infected with angular leaf spot were grown the previous summer. In these tests, extending over 2 winters, 10 or 12 soil samples, each consisting of slightly less than $\frac{1}{2}$ tumbler of soil, were collected each time from a plot and tested on separate tobacco leaves. In 1940–41, 182 samples were collected between November 2 and April 17 on 15 different days from a plot on which tobacco had been heavily infected with *Bacterium angulatum* the summer of 1940 and planted with rye in the fall. Thirty-seven soil samples collected on 9 different occasions caused angular leaf spot. The number of spots ranged from 2 to 300 per leaf. Seventy-two samples collected on 6 dates between May 8 and July 16, 1941, from the same plot all failed to give infection. All of this period was after the cover crop had been plowed under. The following winter the tests were repeated in two plots that had been in continuous tobacco for several years and planted with a rye cover crop during the winter. Angular leaf spot and wildfire were present in the tobacco in 1941 in the first plot and only angular leaf spot in the second. The results obtained were similar to those of the previous year. From the first plot 160 samples were collected on 16 days between August 27, 1941, and March 24, 1942. Of these, 56, or 35 per cent, gave angular leaf spot or wildfire or both. There were a total of 659 angular leaf spots and 14 wildfire spots. The number of spots per leaf ranged from 3 to 250. Sixty collections made between April 13 and June 20, 1942, gave no infection.

From the second plot 180 collections were made between August 27, 1941, and April 21, 1942. Of these, 51, or 28 per cent, gave angular leafspot. A total of about 2023 spots were produced, ranging in number between 1 and

[4] Wolf, F. A. Wildfire of tobacco. N. C. Agr. Exp. Stat. Bull. 246. 1922.

[5] Diachun, Stephen, W. D. Valleau, and E. M. Johnson. Isolation of *Bacterium angulatum* from overwintered tobacco field soil. Phytopath. 32: 2–3. 1942.

[6] Water soaking is done by directing a stream of water preferably against the lower side of the leaf when the stomata are open. In the present study, it was done with a 10- or a 40-cc. injection syringe filled with water and the needle held about an inch from the leaf surface. If the stomata are open, the tissues water-soak quickly and water soaking disappears within about 15 minutes, leaving no sign of injury.

1700 on individual leaves. These tests afforded positive proof that both *Bacterium angulatum* and *Bact. tabacum* survive in field soil over winter, but give no suggestion as to the means of survival in the soil.

CARRY-OVER IN ASSOCIATION WITH PLANT ROOTS

With the idea in mind that certain cover crops might tend to depress the overwintering of the organisms, 6 small plots were laid out, of which 5 were inoculated on October 11, 1941, with cultures of *Bacterium angulatum* and one with crushed dried leaves infected with angular leaf spot. Three plots were seeded with crimson clover, vetch, and wheat, respectively, and 3 left fallow.

TABLE 1.—*Number of angular leaf spots produced by pouring upon a water-soaked tobacco leaf a mixture, with water, of 10 small soil samples from a plot. The plots were inoculated October 11, 1941, as indicated*

When tested	Plots with cover crops			Fallow plots		
	Crimson clover	Hairy vetch	Winter wheat			
	Soil inoculated with					
	2 pure cultures				Mixed cultures	Infected dried tobacco leaves
1941						
Oct. 22	600	400	600	400	400	300
Nov. 14	6	19	67	2	23	0
Dec. 6	17	116	50	1	1	500
Dec. 15	7	15	39	300	0	0
Dec. 23	45	6	146	2	0	2
1942						
Jan. 13	14	69	5	0	0	0
Jan. 22	31	69	16	0	0	0
Feb. 5	66	6	500	1	0	11
Feb. 12	80	180	180	16	9	0
Feb. 19	108	54	60	3	5	53
Mar. 4	31	18	74	0	5	0
Mar. 17	50	72	12	0	0	0
Apr. 2	30	14	11	0	0	0
Apr. 20	33	320	23	0	3	0
May 5	11	9	4	0	0	0
May 23	0	0	0	0	0	0
Totals	1189	1367	1787	725	446	866

On 16 different dates between October 22, 1941, and May 23, 1942, a composite of 10 small samples was taken from each plot and tested together on water-soaked leaves. The results of these inoculations are given in table 1. On every date except the last, *Bacterium angulatum* was isolated in the inoculated tobacco leaves. It is evident from the table that the organism was isolated much more consistently from the plots with cover crops (45 times) than from the fallow plots (20 times). These results suggested, during the winter, the possibility that the organisms were growing on the roots of the crop plants and perhaps also on the roots of occasional weeds that were pres-

TABLE 2.—*Results of inoculation with washed roots of crop plants and weeds grown in soil inoculated with Bacterium angulatum November 11, 1941. Roots collected at intervals between February 19, 1942, and June 4, 1942*

	Crimson clover	Vetch	Wheat	Henbit	Chick-weed	Oxalis	Carda-mine
Number of tests	9	9	10	6	3	2	1
Number positive	6	8	8	6	1	0	0
Total roots tested	45	45	46	23	7	8	2
Number positive	18	22	33	9	2	0	0
Range of number of spots on inoculated leaves	1 to 56	1 to 300	3 to 800	1 to 40	4 to 14	...	...

ent in the fallow plots. Therefore, tests to determine this point were conducted with the roots of plants growing in these plots.

Roots to be tested were dug on 5 different days between February 19, 1942, and June 4, 1942. The roots were washed in running water, and dirt particles were gently rubbed from them with a bit of paper towelling. The roots were then crushed in water and the water poured over the under surface of a water-soaked leaf. Usually, 5 separate root systems of each species were tested on each date. Heavy infection resulted from some of the roots of each of the crop plants in all tests except those made on June 4, when slight infection was obtained from vetch only. At this time the crimson clover and wheat plants were dried and the vetch nearly dead. A summary of these tests is given in table 2. The results indicate that during the winter and spring *Bacterium angulatum* is closely associated with the roots of several plants unrelated to tobacco.

Collections of roots of rye were made from 2 plots on the station farm, in which tobacco was affected with angular leaf spot in 1941, to determine whether roots of a cover crop following tobacco would harbor *Bacterium angulatum* over winter. Collections were made March 11 and 23 and April 7, taking 20 roots on each date from each plot sampled. These were carefully washed as before in running water, crushed, and inoculations made.

The results of inoculations presented in table 3 give proof that *Bacterium angulatum* is maintained overwinter in close association with the roots of rye planted following an infected tobacco crop.

The preceding tests dealt primarily with *Bacterium angulatum*. *Bact. tabacum* appears to live in association with roots in the same way, as indi-

TABLE 3.—*Inoculations from washed roots of rye collected in March and April in a field in which tobacco was naturally infected with angular leaf spot the previous summer*

	Plot 1	Plot 2
Number of tests	3	2
Number of tests positive	3	2
Number of roots tested	60	40
Number of roots positive	12	13
Range in number of spots	1 to 80	1 to 51

cated by the results of inoculations made with washed rye roots collected during late winter from a field in which wildfire was present in tobacco the previous summer. Collections of soil and roots were made March 27, April 15, May 5, and June 1, 1942. *Bact. tabacum* was isolated in tobacco leaves on each occasion, but less frequently as the plants became older. On March 27, 80 roots, and 110 small soil samples, with no attention to whether they contained roots or not, were collected. The soil clinging to the roots was knocked off, making a composite soil sample from every 10 roots. The roots were washed as previously described, and 10 root systems crushed together. Inoculations were then made on water-soaked leaves with soil, soil from roots, and crushed roots. The results presented in table 4 show that *Bact. tabacum* can be isolated from soil samples (which may have contained roots), from soil shaken from roots, but was obtained in greatest abundance from the washed roots. It seems evident that *Bact. tabacum,* as well as *Bact. angulatum,* can live in association with the roots of rye over winter.

A similar test was made with barley roots collected from a field in which angular leaf spot was present the previous summer, and with wheat roots from a field in which both wildfire and angular leaf spot were present the previous summer. The barley roots caused extensive angular leaf spot and the wheat roots caused mixed infection of angular leaf spot and wildfire, on inoculated tobacco leaves.

TABLE 4.—*Inoculations from soil, soil shaken from roots and crushed rye roots collected March 27, 1942, from a field infected with wildfire in 1941*

No. of tests and wildfire spots	Soil	Soil from roots	Roots
Number of tests	11	8	8
Number of tests positive for wildfire	2	8	8
Total number of wildfire spots	2	57	222

CARRY-OVER THROUGH SECOND WINTER IN ABSENCE OF TOBACCO

In the spring of 1943 soil collections containing crimson clover roots were made in a field that was in tobacco in 1941, wheat in 1941–42, and crimson clover in 1942–43. On April 7 and 8, 1943, 15 collections of 5 cores each were used as inoculum. Fourteen of the 15 caused angular leaf spot, with spots ranging from 6 to 500 on inoculated leaves. These results indicate that the bacteria may be found on the roots of cover-crop plants which do not directly follow tobacco in the rotation.

INFECTION FROM WASHED ROOTS OF TOBACCO FROM PLANT BEDS AND FIELD

Infection in plant beds usually does not appear until the plants are well established and are covering the ground. Then it develops only following a protracted wet, cool period. In view of the association found between the bacteria and cover crops during the winter, it seemed probable that the bacteria in the plant bed might first multiply on the roots of tobacco plants be-

fore causing leaf infections. Therefore, plants were collected from 81 plant beds between April 28 and June 2, 1942. The roots from each bed were washed separately, crushed, and used as inoculum on water-soaked leaves. Angular leaf spot resulted from 22 collections, and wildfire from 4. Infections per inoculated leaf ran as high as 2000. Seven of the beds from which root collections caused angular leaf spot appeared to be entirely free from leaf spots at the time the collections were made.

During the summer of 1942 tobacco roots were collected from the field at intervals between June 18 and September 23. The roots were washed and crushed, and water-soaked leaves inoculated. Fifty-seven roots were collected from plants affected with wildfire, of which 42 produced wildfire on inoculated plants. Of 56 roots from plants affected with angular leaf spot, 2 caused wildfire and 44 angular leaf spot. Thirty-eight roots from plants affected with both caused wildfire on 25 inoculated leaves and angular leaf spot on 17. Sixteen plants were collected that appeared to be free from infection, but were in fields where diseased plants could be found. One caused wildfire and 12 angular leaf spot. These tests demonstrate that both organisms are in close association with the roots of tobacco plants in the plant bed and throughout the summer in the field, until the time for sowing cover crops.

PRESENCE OF BACTERIUM ANGULATUM AND BACT. TABACUM IN PROSPECTIVE PLANT BED SITES

The evidence given proves that *Bacterium angulatum* and *Bact. tabacum* can maintain themselves on the roots of tobacco and several crop plants. It would appear therefore that there is no necessary relation between tobacco and the perpetuation of these organisms in a field. Observational evidence in the past has demonstrated that *Bact. angulatum* is nearly always present in burley tobacco plant beds and *Bact. tabacum* sometimes present if weather conditions are such as to make their presence apparent. It, therefore, seemed likely that the organisms could be isolated from old pastures and fence rows, such as are commonly used for making tobacco beds. Tobacco beds in Fayette and neighboring counties usually are placed in a new site each year, although a few farmers set aside a small lot and rotate the beds with bluegrass sod.

Soil collections were made from the sod in the vicinity of recently prepared beds and from fields that had been in pasture for years and in which beds had not been prepared. A soil sample consisted of 5 cores collected with a soil-sampling tube. The core necessarily contained roots of any plants growing at the point of sampling. Usually 10 such samples were collected from a field, making a total of 50 cores. The samples were brought to the greenhouse, stirred in water until the soil particles had separated, and were then poured over a water-soaked tobacco leaf. Collections were made on 38 farms. No infection resulted from 180 samples from 18 farms, but, from the other 20, angular leaf spot resulted from 16 collections of a

total of 200 tested (Fig. 1, A), wildfire from only 2, and wildfire and angular leaf spot from 3.

One field was of particular interest. The bed was located about 4 feet from the fence and was heavily infected with wildfire throughout its length. The remainder of the field had been plowed and prepared for tobacco. It had been in orchard grass-Korean lespedeza pasture for the past 5 years, following a crop of tobacco. Soil cores were collected along the fence row and in the field, and weeds and seedling lespedeza plants were collected in the field away from the bed and on higher ground. Of the 10 composite sam-

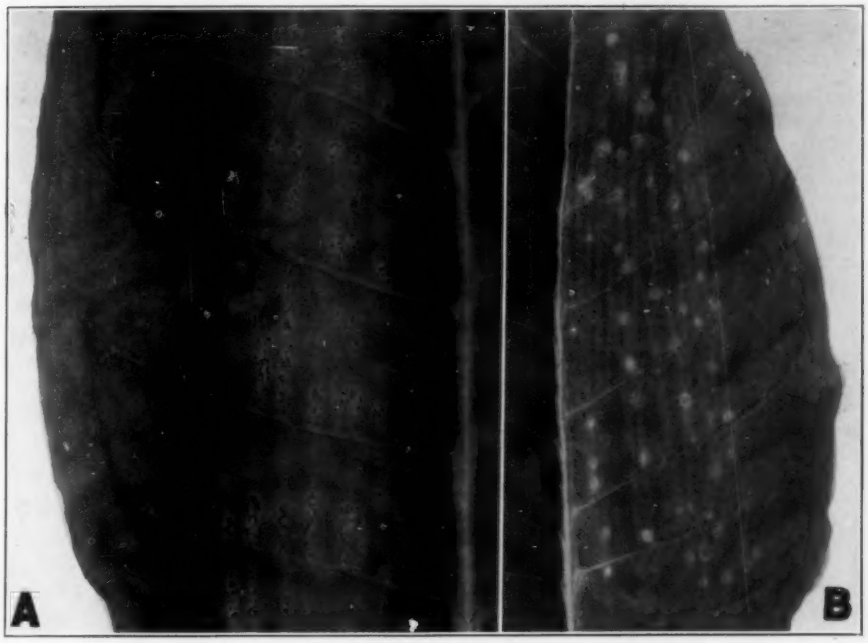

FIG. 1. A. Angular leaf spot resulting from a culture made from a leaf inoculated with field soil containing weed roots. About ½ natural size. B. Wildfire resulting from inoculation of a tobacco leaf with a composite of 5 soil cores collected along a fence row near a wildfire-infested plant bed. About ½ natural size.

ples of soil collected along the fence row, 9 gave wildfire spots ranging in number from 1 to 75 (Fig. 1, B). Two of these caused angular leaf spot also, and 1 sample caused only angular leaf spot. Ten composite soil samples, collected in the plowed field from spots with weeds, gave wildfire alone from 2, wildfire and angular leaf spot from 5, and angular leaf spot from 1. Weeds were dug from the field and fence row, roots were washed, and species tested separately. Angular leaf spot was obtained from roots of ragweed, giant ragweed, white clover, vermifuge and oxalis, and wildfire from roots of Korean lespedeza, shepherd's-purse, white clover, ragweed, chickweed, and orchard grass. At the time these collections were made, some growers were beginning already to set tobacco.

These results prove that *Bacterium tabacum* and *Bact. angulatum* are present in the soil in pastures at the time plants become infected in the plant bed, and that they also are present in association with plant roots in fields plowed and ready to set to tobacco.

COLONIES ON ROOTS

From the evidence presented, it appears that the pathogenic leaf-spot organisms are closely associated with the roots of tobacco, other crop plants,

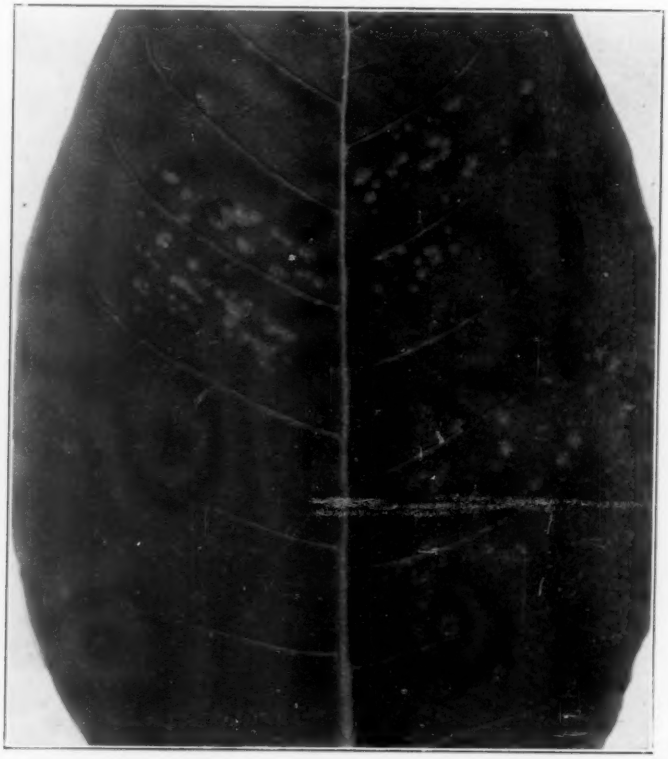

FIG. 2. Wildfire on a tobacco leaf resulting from inoculation with 4 bits of tobacco roots, each bearing a bacterial colony. Each bit of root was crushed separately in water and poured over the under surface of two water-soaked interveinal spaces.

and some weeds. If tobacco roots are removed from a plant bed in an area where plants are injured by either wildfire or angular leaf spot, or if roots are taken from plants in a field affected with one or the other of these diseases and the small rootlets washed and examined with a microscope at low power, colonies of bacteria can be found growing on the surface of the rootlets. Some of the colonies appear soft and watery because motile bacteria can be seen swimming from the surface, while other colonies appear firm, with the surface bacteria remaining in place. To determine whether or not these were colonies of pathogenic bacteria, a very small bit of root bearing a colony was removed, crushed in water, and used as inoculum on a small

water-soaked area of a leaf. When the roots were from plants affected with angular leaf spot, root colonies produced angular leaf spot, and, when from plants with wildfire, this disease resulted from inoculation with root colonies. In all, 59 colonies were selected for test from roots of plants from a tobacco

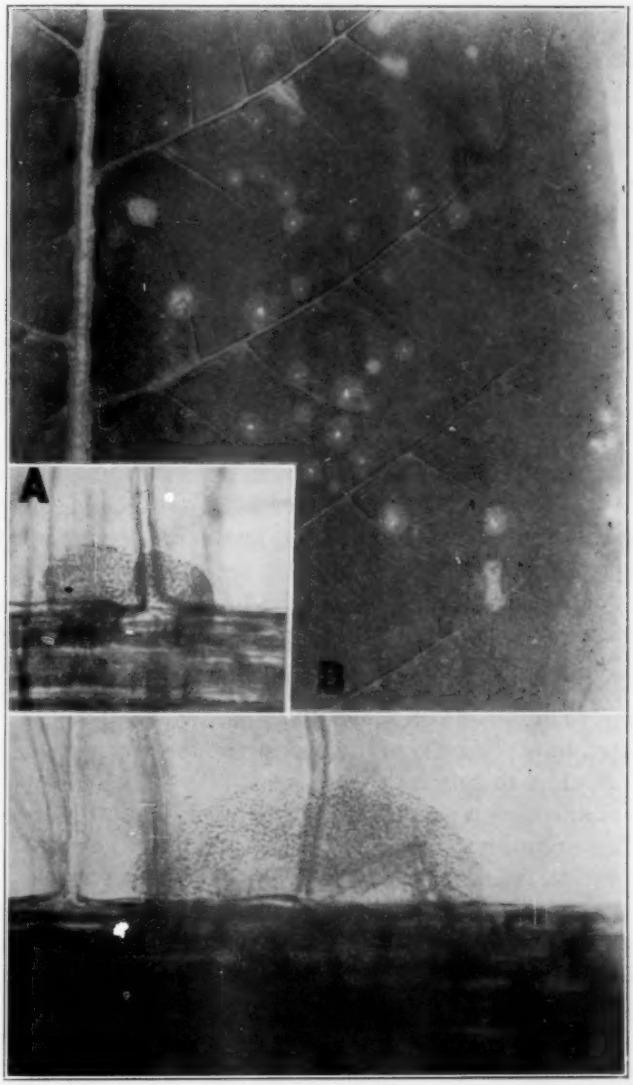

FIG. 3. A. A colony of *Bacterium tabacum* growing on the surface of a wheat rootlet. About ×200. B. Infection produced on a tobacco leaf using colony shown in A as inoculum. C. A colony of *Bact. angulatum* on the surface of a wheat rootlet. About ×200.

bed with wildfire. Fifty of the colonies caused wildfire, with an average of 57 wildfire spots for each inoculation (range 1 to 300) (Fig. 2). Fifty-three colonies from roots of plants from a bed affected with anglar leaf spot were

tested. Forty-six caused angular leaf spot, with an average of 46 spots per leaf (range 2 to 200).

Wheat had proved to be a crop on the roots of which both species of bacteria overwinter. As the seeds are readily surface-sterilized (2 hours in 1¼ per cent solution of NaClO), and germinate quickly, wheat was used in further studies of the colonies on roots. Treated wheat seeds were germinated in a damp chamber. When the roots were developed, they were inoculated by dipping into an aqueous suspension of *Bacterium tabacum* or *Bact. angulatum* containing 1 to 2 million cells per cc. After inoculation, the plants were placed on sterile moist paper toweling in Petri dishes. In from 2 to 5 days, colonies could be found on the roots, usually in the region of the root hairs. The colonies were sometimes tenacious and persisted on the roots even after being mounted in water and left for more than 24 hours (Fig. 3, A and C). A total of 96 wheat roots were inoculated with *Bact. tabacum*. Colonies were found on 85 of them. Forty root systems bearing colonies were used as inoculum, and all produced typical wildfire spots on inoculated tobacco leaves. On 66 noninoculated root systems examined, only 1 colony was found. This was obviously a contamination. Twenty noninoculated root systems failed to cause wildfire when used as inoculum.

When individual colonies were removed on a short bit of a wheat root inoculated with *Bacterium angulatum* or *Bact. tabacum*, crushed in water and used as inoculum on tobacco leaves, angular leaf spot or wildfire developed, depending on the organism being tested (Fig. 3, B). These tests leave little doubt that the colonies observed on the wheat roots were those of the two pathogens.

DISCUSSION

In the early studies on wildfire and angular leaf spot, various hypotheses were advanced to explain the overwintering of the organisms and plantbed infection. Most of these were such as would be suggested by a superficial knowledge of the diseases and were not based on actual knowledge of the habits of the organisms. Recently, Reid and co-workers[7] advanced the hypothesis that because the common nonpathogenic *Pseudomonas fluorescens*, and the pathogenic *Bacterium angulatum* and *Bact. tabacum* show antigenic identity, and because *P. fluorescens* could be found on the leaves of common crop plants and in all tobacco bed soils, this organism was a potential danger to tobacco. They state "the authors consider that serological techniques measure the sum of the morphological and physiological characteristics (including pathogenicity) of the germ plasm of bacterial cells." It probably would be generally admitted that serological tests could be used to prove the identity of certain chemical compounds in two species of organisms, but the assumption that antigenic identity proves genetic identity of bacterial cells is one that would be difficult to substantiate. No proof, other

[7] Reid, J. J., J. Naghski, M. A. Farrell, and D. E. Haley. Bacterial leaf spots of Pennsylvania tobacco. I. Occurrence and nature of the microorganism associated with wildfire. Penn. Agr. Exp. Stat. Bull. 422. 1942.

than serological tests, was presented by these authors to support their assumption of identity.

The present writers give evidence to show that the tobacco leaf-spot phase of the life cycle of these two parasitic organisms is more or less accidental and is probably not essential to their perpetuation. They are not, therefore, primarily tobacco pathogens, but are organisms apparently adapted to a life on the surface of small rootlets of several plants, both weed and crop. It has been shown that the organisms are present on the roots of weeds and pasture plants at the time tobacco plants are developing in the bed, that they are present on the roots of tobacco plants in the bed, that they may be isolated from the roots of tobacco plants growing in the field throughout the summer, and that they are present on the roots of cover crops, sowed following harvest, throughout the winter and until the roots die the following spring.

If, during the period the plants are in either bed or field, the leaves become water-soaked, either by the internal process or from outside causes such as driving rain, and the pathogenic bacteria are splashed or otherwise carried to the water-soaked areas, the bacteria enter immediately, colonies develop, and leaf spots follow. In several years of inoculation work on young plants in the bed, on green house plants, and on plants in the field, we have failed to find any but physiologically very young or very old leaves, which could not be water-soaked (under proper light conditions), and infected by pouring a suspension of bacteria over the water-soaked surface. In all instances, spots typical of the organisms used (*Bacterium angulatum* or *Bact. tabacum*) developed in the usual time. It may be stated, therefore, that the presence or absence of the diseases in the plant bed, if weather conditions have been favorable, depends not on the nutrition of the plant, as Reid *et al.* claim, but on the presence or absence of the organisms on the roots of plants. Considering the persistence of the pathogenic organisms in the soil, which has been demonstrated in the present studies, it seems unnecessary here to assume that the pathogenic organisms are being derived, in a relatively short period of time, from the nonpathogenic *Pseudomonas fluorescens*. If this is ever proved to occur, then the rate of change and its frequency must be studied and demonstrated before it can be concluded that *P. fluorescens* is a danger in old tobacco-growing areas.

The present study has demonstrated that the pathogenic bacteria form colonies, on the roots of plants, which can be found by microscopic examination and that, upon isolation of the bit of root, typical leaf spots can be produced on tobacco leaves, if it is used as inoculum. The fact that the bacterial leaf-spot diseases of tobacco are found in every country where tobacco is grown suggests that these pathogenic bacteria probably are rather generally distributed over the world. It would not be surprising, therefore, if the very similar colonies photographed by Linford[8] on the roots of maize and

[8] Linford, M. B. Methods of observing soil flora and fauna associated with roots. Soil Science 53: 93–103. 1942.

lettuce were similar to or identical with *Bacterium tabacum* and *Bact. angulatum* or were a pathogen capable of causing a leaf disease on other species of plants than tobacco.

SUMMARY

Colonies of *Bacterium tabacum* and *Bact. angulatum* are found in nature on the roots of pasture and crop plants and can apparently maintain themselves indefinitely in this way. Soil collections, presumably containing roots bearing colonies of the pathogenic organisms, frequently cause wildfire or angular leaf spot in the usual time after inoculations are made, thus demonstrating that the organisms are present in the pathogenic form in the soil and apparently persist in this form. Colonies of bacteria can be found on rootlets of infected tobacco plants from the bed or field, which, when used as inoculum, produce the disease present on the original plant. There appears to be no reason for assuming that outbreaks of either angular leaf spot or wildfire arise other than from colonies of the respective pathogenic organisms already present in the soil before outbreaks occur.

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THE USE OF ETHYL MERCURY PHOSPHATE FOR TREATING TOMATO SEED IN NEW JERSEY[1]

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INTRODUCTION

For many years various seed treatments have been used to destroy organisms carried on or within the seed. Chemical compounds, such as copper sulphate, mercuric chloride, and Semesan, have been used for surface disinfection of tomato seeds with a considerable degree of success (2). Hot-water treatment, since it is effective against internal infection, also has been and still is recommended by some workers (1, 3).

Most of these treatments have one important disadvantage in that they do not protect the seed against recontamination after it has been treated. The introduction of organic mercury compounds, particularly New Improved Ceresan (5% ethyl mercury phosphate), has supplied the seedsman with a treatment which effectively disinfects the surface of the seed (6) and leaves a coating of the chemical, which to some extent protects the very young seedling against pre-emergence damping-off caused by soil organisms. As a result of the effective surface disinfection obtained with ethyl mercury phosphate it has come into rather general use. This has led to the necessity of working out efficient and economical methods of treatment particularly for those seedsmen and canners who handle large quantities of seed. The experiments here described deal chiefly with studies of such treatments.

A large quantity of certified tomato seed is produced each year in New Jersey, and a considerable portion of it is treated with some disinfectant to control surface-borne organisms. This is done chiefly to comply with the regulations of various foreign countries to which the seed is shipped and to meet the requirements for the production of State-certified tomato plants grown in Georgia for shipment to the North.

Bacterial spot (*Phytomonas vesicatoria* (Doidge) Bergey *et al.*) is not common on tomatoes in New Jersey. Bacterial canker (*Phytomonas michiganense* (E.F.S.) Bergey *et al.*) is found occasionally, but it, ordinarily, is not a factor to be reckoned with in treatment of tomato seed, since all certified seed produced is taken from fields certified for freedom from that disease. Septoria leaf blight (*Septoria lycopersici* Speg.) occurs to some extent in some years, but is not of major importance in most seasons. Bacterial wilt (*Phytomonas solanacearum* (E.F.S.) Bergey *et al.*) and anthracnose (*Colletotrichum phomoides* (Sacc.) Chester) occur each year, but do not

[1] Cooperative investigation between the U. S. Department of Agriculture, the agricultural experiment stations of New Jersey, Purdue University (Ind.), Georgia, the Georgia Coastal Plain Experiment Station, and the Georgia Department of Entomology.

[2] Formerly, Agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

appear to be seed-borne. Early blight (*Alternaria solani* (E. and M.) J. and G.) occurs commonly every season, and, from the point of view of both the plant grower and seedsman, is by far the most important disease in tomatoes. Massie (4) and Samson (5) have reported cases of infection of tomato seed by *A. solani*, but this disease rarely is seen on the fruits in New Jersey, and in plating perhaps 75,000 seeds surface-sterilized by various means, neither *Alternaria solani* nor any other tomato pathogen was ever encountered. It, therefore, appears that under New Jersey conditions the efficient surface disinfection of tomato seed generally will insure a high degree of protection against seed transmission of pathogenic bacteria and fungi.

MATERIALS AND METHODS

Throughout the experiments reported in this paper all tomato seed were treated at the rate of 1 lb. of dry seed or 1 qt. of wet seed (recovered from pomace left after fruit is processed for the manufacture of tomato juice) per gallon of treating solution. Except where temperatures are specifically stated, all solutions were made with tap water at a temperature of approximately 60° to 65° F. The standard 1–24,000 solution of ethyl mercury phosphate was prepared by dissolving 1 g. of New Improved Ceresan in 1200 cc. of water. Where dusts were applied the amounts recommended by the manufacturer were used. After thorough shaking of seed and dust, all excess was screened off from the seed.

All seeds were plated on potato-dextrose agar (1.8 per cent agar) and were held seven days before final counts of contaminated seeds were made. All of the germination data recorded are for germination in soil, since quantities of chemicals, too small to interfere with germination in soils, often cause marked retardation of germination on blotters. In each instance the location and type of germination medium have been recorded.

Germination of Tomato Seed Treated with Ethyl Mercury Phosphate as Compared with Seed Treated with Other Standard Seed Treatments

In studying seed treatment with ethyl mercury phosphate, preliminary tests were made to compare its effect on germination with other standard seed treatments. These tests included the following treatments; corrosive sublimate 1–3000 for 10 minutes, followed by a 30-minute wash in running water before drying; ethyl mercury phosphate 1–24,000 for 5 minutes, then dried immediately; ethyl mercury phosphate 1–24,000 for 5 minutes, dried immediately, then treated with either red copper oxide dust or with Vasco-4 dust; ethyl mercury tartrate 1–16,000 for 5 minutes, then dried immediately; mercurous oxide on bentonite 1–32,000 for 5 minutes, then dried immediately; metallic mercury on lampblack (dust); and hot water (55° C. for 10 minutes). All seed was stirred vigorously, while submerged in the treating solution, to be sure that all aggregates of seeds were broken up and all seed surfaces brought in contact with the treating solution. These trials

were conducted in 1938 and 1941, and 300 to 500 seeds per treatment were planted. New Jersey certified seed of a strain of Marglobe tomato was used. The seeds were planted in the greenhouse in a Sassafras sandy loam soil. The results from all trials showed that there was no significant difference in germination between any of the treatments and the untreated checks. In 1941 samples of the seed used in the experiments were also tested to determine the degree of surface disinfection resulting from the various treatments. With the exception of the seed treated with hot water, where 11 per cent of contamination occurred, all of the treated samples showed 99 to 100 per cent of the seed free from surface contaminants. The results with ethyl mercury phosphate are in agreement with those of Samson (6) who reports that a 1–32,000 solution of 5 per cent ethyl mercury phosphate gave complete surface disinfection of tomato seed and did not reduce the germination below that obtained from seed treated with 1–3,000 solution of mercuric chloride.

The results of all the comparative tests mentioned above have confirmed the preliminary data, which indicate that ethyl mercury phosphate is an effective surface disinfectant for tomato seed and when used in the proper concentrations is not likely to cause any serious reduction in germination.

Use of Dyes for Seed Treatment

Since certain seedsmen add dyes to the treating solution to distinguish treated from untreated seed, and since many dyestuffs have fungicidal and germicidal properties, seeds treated with various concentrations of safranine A, methyl violet 2B, brilliant-green B, and chrysoidine 3R plus safranine A were plated on potato-dextrose agar to determine whether or not the dyes alone might be of value as seed disinfectants. Of these dyes only brilliant-green B appeared to have sufficient germicidal strength to give adequate disinfection; and, in order to obtain satisfactory disinfection with it, a solution of such great concentration was necessary that the cost was prohibitive.

Effect of Repeated Use of Solutions of Ethyl Mercury Phosphate in Seed Treatment

Where seed is treated in large quantities there has been a question as to whether the solution would retain its effectiveness if used for the treatment of more than one lot of seed. To test this, successive lots of seed were treated in the same solution and samples of the respective seed lots were then plated to determine the amount of surface contamination still present. The seed was treated for 5 minutes at the rate of 1 lb. of dry seed to 1 gal. of solution, and then removed and spread out to dry. The results of these tests (Table 1) indicate that only a single lot of seed should be treated and the solution should then be discarded.

Effect of Concentration of the Treating Solution on Disinfection and Germination of Seed

Three series of tests were conducted to determine the concentration of ethyl mercury phosphate solution necessary for adequate surface disinfec-

TABLE 1.—*Results of treating successive lots of tomato seed in the same solution of ethyl mercury phosphate, 1–24,000*

Seed lot	Per cent of seed free from contamination	
	First test[a]	Second test[b]
1st	97.5	100
2nd	91.0	97
3rd	71.1	91
4th	.....	47

[a] Treatments made by a commercial seedsman.
[b] Treatments made in the laboratory at 60° F.

tion with a minimum reduction in germination. From the results of the first test (Table 2) it was evident that concentrations greater than 1–20,000 would seriously interfere with germination of the seed. When the greater

TABLE 2.—*Germination of seed treated for 10 minutes with various concentrations of ethyl mercury phosphate at the rate of one pound of dry seed to one gallon of solution. Seed planted in flats of sassafras sandy loam in the greenhouse*

Concentration	Germination	Number days until rows of plants were clearly visible
1st planting[a]	*Per cent*	
1–12,000	61	17
1–16,000	62	15
1–20,000	82	13
1–24,000	84	13
Untreated check	74	12
2nd planting[b]		
1–12,000	63	15
1–16,000	68	13
1–20,000	87	11
1–24,000	91	10
Untreated check	88	9

[a] April 1941.
[b] June 1941.

concentrations were applied the percentage of germination was reduced by as much as 20 to 25 per cent as compared with nontreated seed, and germination was delayed from 3 to 6 days.

TABLE 3.—*Results of treating tomato seed for 10 minutes with various concentrations of ethyl mercury phosphate at the rate of one pound of dry seed to each gallon of solution*

Concentration of solution	Per cent free from contamination	
	1st series	2nd series
1–16,000	.....	100
1–20,000	100	.....
1–24,000	100	100
1–28,000	99	.....
1–32,000	99	100
1–40,000	.....	100
1–48,000	.....	98
Untreated check	0	0

The second and third tests (Table 3) showed that it was not necessary to have solutions of concentrations greater than 1–24,000 in order to secure complete surface sterilization. Since a 1–24,000 solution gave adequate surface sterilization without interfering with germination other than to delay it by approximately one day, this concentration was used in all subsequent experiments.

Length of Exposure to Treatment

Although a 5-minute treatment in the laboratory gave perfect control of surface contamination, in each lot of commercially treated seed a small percentage was contaminated. It seems probable that this is because the centrifugal "wringers" used in commercial seed treating throw off the solution before sufficient mercury has been adsorbed. Two methods of overcoming this difficulty have been found to be entirely satisfactory. Either the exposure can be increased to 10 minutes or the sacks of seed can be left to drain for 20 to 30 minutes before they are placed in the "wringers." When a 1–24,000 solution is used, neither of these practices causes injury to the seed.

Temperature of the Treating Solution

In treating commercial seed in New Jersey, tap water or water drawn from streams is used to make up the solutions and the temperature varies according to the weather at the time of treating. To determine the influence of the temperature of the treating solution on the effectiveness of the treatment, seeds were treated in 1–24,000 ethyl mercury phosphate at temperatures of 43°, 60°, and 80° F. for 5 minutes and 10 minutes, both with and without a draining period before they were placed in the centrifuge. The results of 3 experiments (Table 4) indicated that the temperature of the solution is not of primary importance, since the seeds treated in the solution at 43° F. were as free from surface contamination as those treated in the solution at 80° F. and the germination of the seed treated at the higher temperatures was not significantly lower than that of seed treated at 43° F.

TREATMENT OF FRESHLY EXTRACTED *vs.* DRIED SEED

During wet weather the cost of drying large quantities of seed is very great, and some seedsmen would like to eliminate, if possible, one drying operation. Therefore, experiments were conducted to determine the comparative effectiveness of the treatment of freshly extracted, moist seed, and of seed that had been dried before treatment. In 1938 tomato seed was extracted by fermenting for 36 hours and then washing in water. The seed was divided into 2 lots, one treated immediately, the other dried for 7 days before treating. As shown in table 5, treating immediately after extraction did not reduce the germination of the seed to any appreciable extent, although treating with ethyl mercury phosphate has been shown to retard germination one or two days. Platings showed that all of the lots of treated seed were free from surface contamination.

TABLE 4.—*Influence of temperature of the treating solution upon the effectiveness of seed treatment with 1–24,000 ethyl mercury phosphate*

Length of exposure to treating solution	Drained 30 minutes before centrifuging	Temperature of treating solution	Free from contamination[a]	Germination after 14 days[b]
		Degrees F.	*Per cent*	*Per cent*
1st Experiment				
5 min.	No	43	100	85
5 "	"	60	100	84
5 "	"	80	100	81
5 "	Yes	43	100	82
5 "	"	60	100	87
5 "	"	80	100	85
10 "	No	43	100	80
10 "	"	60	100	84
10 "	"	80	100	81
10 "	Yes	43	100	77
10 "	"	60	99	85
10 "	"	80	100	87
Untreated check	.....	.....	0	79
2nd Experiment				
5 min.	No	45	100	84
5 "	"	60	100	82
5 "	"	80	100	82
10 "	"	45	98	80
10 "	"	60	100	85
10 "	"	80	99	79
Untreated check	.....	.....	0	70
3rd Experiment				
5 min.	No	50	100	84
5 "	"	70	99+	83
5 "	Yes	50	99+	81
5 "	"	70	100	85
10 "	No	50	100	84
10 "	"	70	100	84
Untreated check	.....	.....	0	81

[a] 300 seeds from each lot plated.
[b] 500 seeds from each lot planted in flats of soil in the greenhouse.

TABLE 5.—*Treatment of tomato seed with ethyl mercury phosphate, with ethyl mercury tartrate, or with $HgCl_2$ immediately after extraction, and after thorough drying*

Time of treatment	Treatment	Germination after 14 days[a]	Free from contamination[b]
		Per cent	*Per cent*
First experiment			
Immediately after extraction	Ethyl mercury phosphate 1–24,000	91.1	100
" " "	$HgCl_2$ 1–3,000	92.1	100
After drying	Ethyl mercury phosphate 1–24,000	92.5	100
" "	$HgCl_2$ 1–3,000	93.0	100
Check	None	91.3	5
Second experiment			
Immediately after extraction	Ethyl mercury phosphate 1–24,000	98.1	100
" " "	Ethyl mercury tartrate 1–15,000	97.1	100
" " "	$HgCl_2$ 1–2,500	96.8	99
Check	None	96.4	11

[a] 750 seeds from each lot planted in flats of soil in the greenhouse.
[b] 250 seeds from each lot plated.

A second lot of seed, also extracted by fermentation, was treated immediately after extraction. Platings showed that all lots of the treated seed were free from surface contamination. Table 5 shows that treatment immediately after extraction did not reduce the percentage of germination of the seed. In this experiment ethyl mercury tartrate was tested for comparison with ethyl mercury phosphate and gave perfect control of surface contamination without interfering with germination of the seed. However, since it did not appear to have any advantage over the commercially-available ethyl mercury phosphate it was not tested further.

Effect on Germination of Type of Container Used for Storing Seed Treated with Ethyl Mercury Phosphate

A third lot of seed, extracted by fermentation, was treated with a 1–24,000 solution of ethyl mercury phosphate or with a 1–3,000 solution of $HgCl_2$, part immediately after extraction and part after being thoroughly dried and stored for two weeks. Each lot was then divided into 3 parts, one of which was stored in ordinary paper bags, one in tight cardboard containers, and one in sealed jars. Plantings of 500 seeds each, made at intervals over a period of 2 years showed (Table 6) that the treatments, whether made immediately after extraction or after drying, do not seriously affect the germination of the seed. It also appears that seed treated in either the freshly extracted or the dried state can be stored in air-tight containers for periods up to 2 years without seriously affecting its germination. After 2 years the germination of all seed lots was considerably lower than at the beginning of the experiment. This reduction in germination was much more marked in the seed stored in paper bags than in either of the other types of containers. The laboratory in which the seeds were stored was perhaps warmer and more humid than most seed storage rooms, and it is probable that the reduction in germination was greater than would occur in commercial seed storage.

Since the above tests with freshly extracted seed were all made in the laboratory and involved only small lots of seed, it seemed advisable to repeat part of the experiment using larger amounts of seed and making the treatments under conditions comparable with treatments made by commercial seedsmen. A quantity of pomace was, therefore, obtained, a part of which was extracted immediately and a part put aside to ferment for 24 hours before being washed out. Each of these lots was again divided into 2 parts, one for treating immediately and the other for treatment after having been thoroughly dried. Two identical experiments were conducted on this phase of the problem.

Since the treatments were made at a cannery where highly sensitive scales were not available, concentrations slightly different from those ordinarily recommended had to be used (ethyl mercury phosphate at the rate of 1–20,480 instead of 1–24,000, and $HgCl_2$ at the rate of 1–3,072 instead of the usual 1–3,000).

The data in table 7 show that, with the exception of 2 or 3 unexplainable instances in one experiment neither of these chemicals seriously interfered with the germination of the seed, regardless of whether they were applied immediately after extraction or after the seed had been thoroughly dried. This was also true with regard to the method of extraction, since no appreciable differences were observed in the germination of the seed extracted by the two methods.

TABLE 6.—*Germination of tomato seed treated immediately after extraction and after thorough drying, when stored in various types of containers*

Type of container	Time of treatment	Treatment	Germination after storage for		
			2 weeks	1 year	2 years
			Per cent	*Per cent*	*Per cent*
Paper bags	Immediately after extraction	Ethyl mercury phosphate 1–24,000 for 5 minutes	89.3	86.3	43.4
Paper bags	Immediately after extraction	$HgCl_2$ 1–3,000 for 5 minutes	84.0	86.3	50.0
Paper bags	After drying	Ethyl mercury phosphate 1–24,000 for 5 minutes	86.2	83.0	53.4
Paper bags	After drying	$HgCl_2$ 1–3,000 for 5 minutes	88.3	87.7	42.6
Paper bags	Check	None	83.7	98.7	30.2
Cardboard cartons	Immediately after extraction	Ethyl mercury phosphate 1–24,000 for 5 minutes	87.1	87.0	70.8
Cardboard cartons	Immediately after extraction	$HgCl_2$ 1–3,000 for 5 minutes	86.3	86.7	72.0
Cardboard cartons	After drying	Ethyl mercury phosphate 1–24,000 for 5 minutes	87.6	88.0	82.4
Cardboard cartons	After drying	$HgCl_2$ 1–3,000 for 5 minutes	88.8	89.3	70.0
Cardboard cartons	Check	None	86.1	90.0	55.8
Sealed jars	Immediately after extraction	Ethyl mercury phosphate 1–24,000 for 5 minutes	87.6	84.7	77.2
Sealed jars	Immediately after extraction	$HgCl_2$ 1–3,000 for 5 minutes	87.2	86.7	42.2
Sealed jars	After drying	Ethyl mercury phosphate 1–24,000 for 5 minutes	91.2	83.0	50.8
Sealed jars	After drying	$HgCl_2$ 1–3,000 for 5 minutes	88.1	82.0	73.6
Sealed jars	Check	None	98.9	84.7	61.2

In 1941, numerous platings also were made of seed treated for 10 minutes in a 1–24,000 solution of ethyl mercury phosphate by a commercial seedsman. Part of the seed was treated at the time of extraction and part several weeks later. All lots tested were free from surface contamination.

These experiments indicate that treatment of tomato seed with either 1–3,000 $HgCl_2$ or 1–24,000 ethyl mercury phosphate can be carried on either at the time of extraction or at a later date, regardless of the method of extraction, with equally successful control of surface contamination and

with equal assurance that the treatment will not seriously interfere with the germination of the seed.

TABLE 7.—*Germination of tomato seed under conditions comparable with treatment by commercial seedsmen*[a]

Method of extraction and treatment applied	Germination			
	First experiment		Second experiment	
	Greenhouse, 18 days	Field, 19 days	Greenhouse, 18 days	Field, 19 days
	Per cent	*Per cent*	*Per cent*	*Per cent*
Not fermented				
Treated immediately; ethyl mercury phosphate 1–20,480 for 5 minutes	68.6	49.6	99.2	64.0
Treated immediately; $HgCl_2$ 1–3,072 for 5 minutes	86.0	55.0	93.4	71.3
Dried, treated later; ethyl mercury phosphate 1–20,480 for 5 minutes	90.2	62.8	92.0	82.0
Dried, treated later; $HgCl_2$ 1–3,072 for 5 minutes	85.4	58.8	94.8	64.4
Check—not treated	85.8	65.6	80.2	55.3
Extracted by fermentation				
Treated immediately; ethyl mercury phosphate 1–20,480 for 5 minutes	74.2	68.8	87.2	78.6
Treated immediately; $HgCl_2$ 1–3,072 for 5 minutes	88.4	58.0	90.8	76.8
Dried, treated later; ethyl mercury phosphate 1–20,480 for 5 minutes	89.4	60.6	85.4	77.0
Dried, treated later; $HgCl_2$ 1–3,072 for 5 minutes	90.0	45.2	92.4	69.8
Check—not treated	86.2	64.4	79.0	72.4
Standard error	9.129±	18.280±	8.811±	12.229±

[a] All treated lots were free from surface contamination.

SUMMARY

Ethyl mercury phosphate has proved as effective as mercuric chloride as a means of surface disinfection for tomato seed and it possesses the added advantage that a residue of the organic mercury compound may be left safely on the seed coat. This tends to prevent recontamination of the seed and affords a certain degree of protection against seedling damping-off.

Concentrations of ethyl mercury phosphate greater than 1–20,000 cause a reduction in the percentage of germination as well as a slowing up of the rate of germination. Since this concentration controls surface contamination as well as stronger concentrations without seriously interfering with the germination of the seed, it can be recommended for general use.

Solutions of ethyl mercury phosphate become less effective with repeated use and should be discarded after one treatment.

A 5-minute treatment has given perfect results in laboratory tests but when commercially treated seed is centrifuged immediately after treatment a period of 10 minutes will allow greater adsorption of the mercury compound and give more perfect surface disinfection. If the sacks of seed are

allowed to drain for 20 to 30 minutes before centrifuging, a 5-minute treatment will probably be effective.

The treating solution is equally effective at temperatures from 43° to 80° F. Tap water or water drawn from streams during the growing season is satisfactory for making up the solution.

Tomato seed can be treated in solutions of the above strength either at the time of extraction or after having been thoroughly dried, and may be stored from one season to the next in any type of container that is satisfactory for nontreated seed.

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EFFICACY OF FUNGICIDAL TRANSPLANTING LIQUIDS FOR CONTROL OF CLUBROOT OF CABBAGE[1]

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The clubroot organism (*Plasmodiophora brassicae* Wor.) is particularly difficult to control in acid soils. Although raising the pH of the soil solution to neutral or slightly alkaline by application of lime is sometimes effective, it is not always possible to maintain the reaction at such points with sufficient constancy to prevent infection (10, 24). Moreover, on acid muck the buffer action of the soil is sometimes such as to preclude any successful control by this method (27).

A number of workers have tried to control the disease in seed beds by applying fungicides broadcast along the row, but results have not been consistently successful (1, 2, 3, 4, 6, 7, 8, 9, 26), and such methods are, of course, applicable only to plant culture in cold frames or greenhouses. In 1927 Preston (13) offered a new approach when he reported reduction of the severity of the disease by adding mercuric chloride to the transplanting liquid in the dibble hole when plants of cabbage (*Brassica oleracea* var. *capitata* L.) were set in infested soil in the field. This method had been tried with some success by Halsted (3) and by Kindshoven (8), but neither of these investigators seems to have found it worthy of general recommendation. Preston (14, 15, 16, 17, 18) presented further results with other compounds, but mercuric chloride was in each case the most effective. Smieton (21) tried with some success pentachloronitrobenzene and trichloronitrobenzene mixed with soil in seed-boxes and in the dibble hole, but mercuric chloride was the most effective treatment with out-of-door plants. In Russia, Rochlin (19) reported that volatile mustard oil was toxic to the clubroot organism and that a crude extract of leaves and stems of black mustard (*Brassica nigra* L.) when added to the soil around young plants reduced the amount of infection that occurred. Preston (17) did not secure any beneficial results by watering mustard plants growing in infected soil with 0.05 and 0.0125 per cent allyl isothiocyanate, the sulphur oil most prevalent in mustard leaves. Immersing cabbage seedlings for 3 to 46 hours in the same solutions before setting on infested soil also failed to reduce clubroot infection. He also tried the incorporation into the soil in the dibble hole of 3 compounds: (1) calomel (HgCl); (2) an amalgam in the form of a fine powder

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containing 12 per cent metallic mercury and 6 per cent zinc and 82 per cent calcium carbonate; (3) a powder form of chloronitrobenzene known under the proprietary name of brassican. All of these reduced infection substantially, but none was superior to mercuric chloride in effectiveness. Indine blue, auramine, malachite green, and magnesium sulphate used as dusts in the soil of the dibble hole were of no value. The use of mercuric chloride in the transplanting water, with marked success, has been reported by others in England (11, 20, 22, 23).

The writers conducted the present study in 1938 to 1941. The purpose was to test the efficacy of mercuric chloride and mustard oil as fungicides in the transplanting liquid on mineral and muck soils and to explore the possibility of other compounds that might be used. The work was carried on in the greenhouse at Madison, Wisconsin, and in the field in commercial cabbage areas in Racine and Kenosha Counties, Wisconsin. A preliminary report has already been published (25). Experiments in New York have been reported by Palmer (12).

METHODS AND MATERIALS

Two types of soil—mineral and muck—were used, since cabbage culture is common to both. A field of Clyde silt loam in Kenosha County, known to be severely infested, was used as representing the first type, while most of the trials on muck were made in a tract near Wind Lake, Racine County.

In the early experiments, which included numerous chemicals and dosages, single-row plots of 20 to 30 plants each were employed. The usual transplanting hole was made, the specified amount of liquid applied, and a plant from a healthy seed bed set and covered. After the normal time for recovery and renewed growth, note of injury to the plant was taken from the appearance of the aboveground portion of the plant. Four grades of plant injury were arbitrarily established extending from 0 for no injury to 3 for severe injury. At harvest all plants in the plot were pulled, and each placed in one of 5 classes of disease based on the extent of severity, from no infection to very severe clubbing. On the basis of such grades, which were given equal weight, a disease index for each plot was calculated. The index might vary from 0 for all clean plants to 100 for all severely clubbed plants. Heads were sometimes removed and weighed for the determination of yield.

When large trials on a commercial scale were conducted, the results usually were secured by pulling plants at random in each treatment and determining the disease index. When yields were estimated, blocks of 25 heads were cut at intervals and weights secured.

Chemicals used were all secured from reliable manufacturers.

Preliminary tests with many of the chemicals were made in the greenhouse in order to determine the relative range of toxicity to the host and to the pathogen. Infested muck soil from the Wind Lake area was used in 6-inch clay pots. Six to 10 cabbage plants in the third- to fourth-leaf

stage were used as test plants in each pot. Five ml. of a solution or uniform suspension of the chemical were placed in the small hole made for each plant. Whereas data secured from these tests could not be applied directly to field performance, they did give opportunity to test in a preliminary way the relative value of an unknown in comparison with a substance on which a field reading had been made.

EXPERIMENTAL RESULTS

Comparison of Allyl Isothiocyanate and Mercuric Chloride

In 1938 allyl isothiocyanate and mercuric chloride were compared in field trials on naturally infested Clyde silt loam soil in Kenosha County, Wisconsin. Cabbage plants were set on June 18 with 425 ml. of liquid per plant in single-row plots of 20 plants with 14 replicates of each treatment. Final results, taken on September 29, are presented in table 1. It will be seen

TABLE 1.—*Comparison of clubroot development on cabbage when transplanted with liquid containing allyl isothiocyanate, in various amounts, or mercuric chloride. (Average of 14 replicates per treatment)*

Agent	Concentration	Disease development	Plants headed	Average wt. per head	Yield per acre
	p.p.m.	*Index*	*Per cent*	*Lb.*	*Tons*
None	.....	100.0	0.0	0.0	0.0
Allyl isothiocyanate	50	97.9	7.0	3.2	0.76
	125	92.9	9.8	2.3	0.80
	250	93.4	5.6	1.8	0.35
	500	91.4	17.3	2.1	1.35
	1000	87.3	17.4	2.3	1.45
Mercuric chloride	667	27.2	74.4	4.2	12.35

that mercuric chloride at 667 p.p.m. (1–1500) was very effective in reducing disease development. A large percentage of the plants produced good-sized heads, and a yield of 12.35 tons per acre was harvested. Although in greenhouse trials, reported elsewhere (5), concentrations from 10 to 350 p.p.m. of allyl isothiacyanate in inoculum of spore suspensions prevented infection, very little effective control was secured in the field when concentrations up to 1,000 p.p.m. were used in the transplanting water. Moreover, at the highest concentrations there was considerable injury to the plants.

The appearance of a typical plot treated with mercuric chloride is shown in figure 1. When the plants were pulled and examined, it was found that the chief benefit of the treatment was in the protection of the young rootlets that were produced in abundance from the pericycle of the tap root when the plant was set into moist soil. Ordinarily, the high soil moisture thus provided favored not only root development by the host but spore germination by the pathogen. Thus, heavy club formation occurred immediately around the tap root of the control plant and the feeding capacity of the root system was rapidly impaired. When an effective fungicide was provided in the

transplanting liquid, infection of the plant at this vital locus was largely prevented. Although clubs formed later at various points on the root system, the detrimental effect on plant growth was much less pronounced. In figure 2 are shown a plant from an untreated row and one from a mercuric chloride-treated row, collected at the time of harvest. Many of the numerous clubs on the former had decayed, and the functional feeding roots were few. In the latter a functional root system still remained regardless of clubs formed at various scattered points.

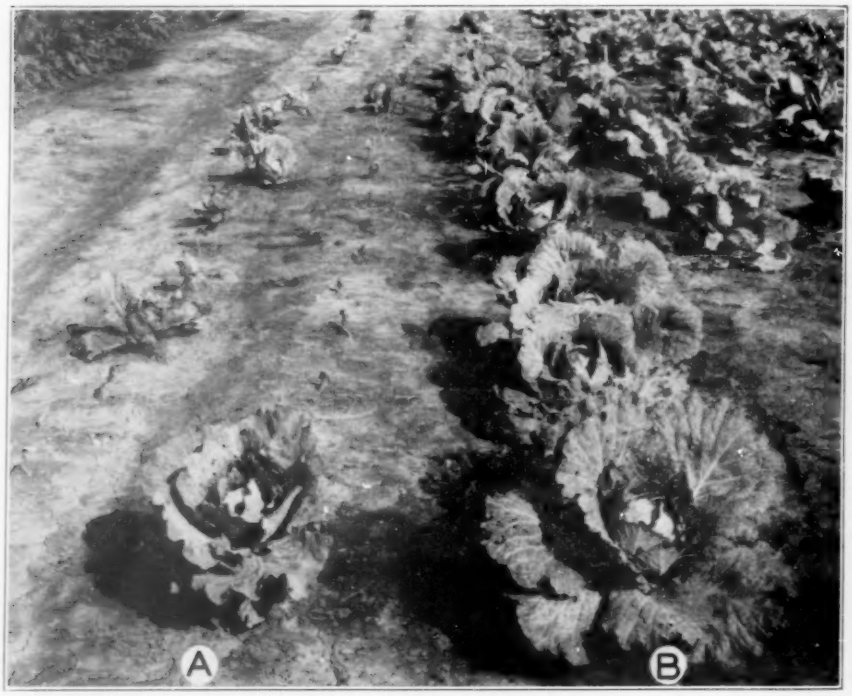

FIG. 1. Effect of use of mercuric chloride on control of clubroot in heavily infested soil. A. Plants, at the end of the season, which had been transplanted with water (425 ml. per plant). B. Plants transplanted with mercuric chloride (1–1500 solution, 425 ml. per plant).

Exploratory Trials With Various Fungicides

In 1939 trials were made with a considerable number of other fungicides added to the transplanting liquid. Most of these were tested in the greenhouse during the winter of 1938–1939. Since the volume of 425 ml. per plant used in the 1938 trials was much higher than that practicable in commercial culture, the dosage was reduced to 125 ml., which was close to the average rate in general use. Various concentrations were used, the range being designed to include at the upper limit a point at which host injury was marked or the cost prohibitive. The trials were made with 26-plant plots on heavily infested muck soil with a pH of 5.7 to 6.1. One plot per

treatment was employed and nontreated rows were included at frequent intervals. The uniformity of infestation is shown by the disease indices of the nontreated plots given in table 2. The inorganic and organic salts of mercury used, the concentrations used, the degree of plant injury, and the disease indices are given in table 2. Results with other organic and inorganic salts are given in table 3. It will be seen that $HgCl_2$, HgCl, HgO, and mercury pentachlorophenolate were superior to other mercury salts,

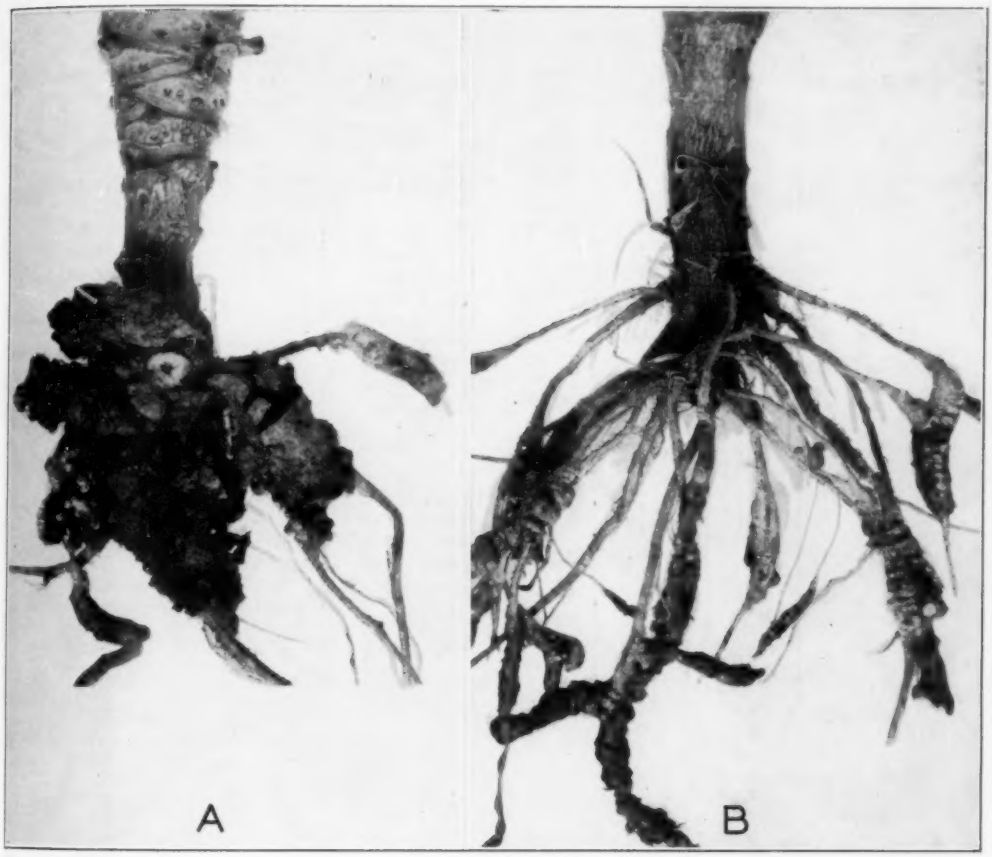

FIG. 2. Comparison of the root systems of untreated and mercuric chloride-treated plants at the end of the season. A. Surviving plant from an untreated row (see figure 1, A) with a few decayed clubs remaining and no functional feeding roots. B. Plant from a treated row. Note that no clubs developed close to the tap root and a well-functioning root system with scattered clubs was formed.

and that little promise was afforded by any of the other materials used. The lack of control with chlorobenzenes was in contrast to more favorable results reported in England for these compounds by Smieton (21). $HgCl_2$ held an advantage over HgCl and HgO because of its solubility in water, while the other two, being low in solubility, had to be kept in suspension by an agitator during application. When HCl was used to facilitate dis-

solving $HgCl_2$, control was about the same, but the acidified solutions produced noticeably greater plant injury.

Relation of Volume and Concentration to Control

In another experiment in 1939 on the same tract of muck mentioned in the foregoing, a series of tests was made in which the amount of chemical

TABLE 2.—*Comparative value of compounds of mercury in reducing severity of clubroot of cabbage when used in the transplanting liquid applied at 125 ml. per plant*

Compound	Grams per liter	Plant injury	Disease index	Compound	Grams per liter	Plant injury	Disease index
$HgCl_2$	10.30	3	25.00	2% ethyl mercury chloride (2% Ceresan)	7.60	2	89.42
	5.17	3	17.31		3.80	0	64.71
	3.48	2	15.28		1.52	0	77.40
	3.10	2	23.86		0.60	0	72.92
	2.90	1	29.55	10% hydroxymercurinitrophenol and 2% hydroxymercurichlorophenol (Semesan Bel)	1.64	0	63.54
	1.74	1	29.29		0.82	0	78.80
	1.50	1	22.22		0.41	0	80.88
	0.87	1	31.59				
	0.35	0	41.62				
	0.14	0	54.17				
$HgCl_2$:HCl	1.74: 8.8	2	16.67				
	1.74: 4.4	3	25.00	Hg. 2, 4, 5-trichlorophenolate	0.60	0	35.00
	1.74: 2.4	2	22.22		0.30	0	46.90
	1.74: 1.2	1	26.14		0.15	0	80.77
	0.87: 4.4	1	43.06		0.08	0	89.42
	0.87: 2.4	0	27.38		0.04	0	84.26
	0.87: 1.2	1	30.97	Hg penta-[a] chlorophenolate	1.60	1	35.00
	0.35: 2.4	1	41.67		0.80	1	36.07
	0.35: 1.2	1	38.09		0.40	0	39.00
HgCl	3.08	0	25.00	Hg ortho-[a] phenylphenol	2.00	0	23.91
	1.54	0	23.91		1.00	0	43.79
	0.77	0	27.05		0.50	0	84.62
	0.38	0	35.79		0.25	0	82.69
	0.19	0	45.19		0.12	0	94.23
	0.10	0	55.21				
HgO	1.30	0	27.11	None	.....	0	91.67
	0.65	0	38.07		.....	0	94.23
	0.32	0	29.35		.....	0	93.27
	0.16	0	50.00		.....	0	98.08
HgS	1.24	0	94.23		.....	0	94.23
	0.62	0	96.64		.....	0	90.38
	0.31	0	89.42		.....	0	87.50
	0.16	0	95.19		.....	0	92.31
					.....	0	88.46
					.....	0	94.23
					.....	0	97.12

[a] Crude product obtained by fusing a mixture containing equivalent quantities of the phenol concerned and mercurous oxide. These mercury phenolates were not available from commercial sources.

and the volume of liquid per plant were varied with each of 3 materials—mercuric chloride, ethyl mercury chloride, and 2, 4, 5-trichlorophenol. Three replicate blocks were set up in each of which the treatments were arranged at random. The results are given in table 4. It will be seen that, in confirmation of the single-plot trials reported in tables 2 and 3, mercuric chloride was significantly superior to the other 2 chemicals. There

was no consistent, significant difference between the 3 volumes of liquid per plant when the amount of chemical remained constant. As the amount of mercuric chloride per plant increased, the disease index at each volume decreased. At 63 ml. per plant there was a significant decrease in index with each increase in total chemical applied. The differences were not significant at 125 ml. and 250 ml. per plant. The results indicated that the

TABLE 3.—*Comparative value of a number of non-mercury compounds in reducing severity of clubroot of cabbage when used in the transplanting liquid applied at 125 ml. per plant*

Compound	Grams per liter	Plant injury	Disease index	Compound	Grams per liter	Plant injury	Disease index
$CaCl_2$	100.0	3	62.50	Pentachlorophenol (Santophen 20)	0.80	1	85.58
	50.00	3	99.04		0.40	1	87.50
	25.00	1	89.42		0.20	0	76.45
$Ca(OH)_2$	128.00	0	72.12		0.10	0	54.55
	64.00	1	86.54		0.07	0	75.00
	32.00	0	61.90	Orthophenylphenol (Dowicide 1)	5.12	1	89.42
$ZnSO_4$	17.4	1	65.34		2.56	1	88.46
	8.7	0	71.56		1.28	1	79.17
	4.3	0	61.96		0.64	0	67.31
$ZnCl_2$	11.3	1	77.88		0.32	0	62.50
	5.6	1	77.19		0.16	0	69.23
	2.8	0	72.73	Chloro-2-phenylphenol (Dowicide 3)	1.28	1	91.35
ZnO	36.4	0	59.02		0.64	0	74.04
	18.2	0	55.17		0.32	0	61.00
	9.1	0	64.89		0.16	0	65.38
$Al_2(SO_4)_3$	85.6	0	97.12		0.08	0	68.27
	42.8	0	91.35	Na chloro-2-phenylphenate (Dowicide C)	5.12	1	79.81
	21.4	0	85.00		2.56	0	73.24
	10.7	0	92.31		1.28	0	62.68
2, 4, 5-trichlorophenol (Dowicide 2)	1.28	2	95.20		0.64	0	58.65
	0.64	2	95.37		0.32	0	53.85
	0.32	2	90.97		0.16	0	68.27
	0.13	0	92.86	2, 4-dinitrochlorobenzene	0.40	2	95.19
	0.05	0	81.73		0.20	1	86.75
Na 2, 4, 5-trichlorophenolate (Dowicide B)	2.56	2	98.08		0.10	0	77.88
	1.28	2	97.12	Chlorobenzene (Brassican)	15.60	0	75.00
	0.64	2	89.42		7.80	0	69.57
	0.32	0	84.41		3.40	0	79.81
	0.16	0	87.50		1.70	0	77.88
	0.08	0	78.85		0.85	0	75.27
2, 3, 4, 6-tetrachlorophenol (Dowicide 6)	0.40	1	92.31		0.42	0	71.15
	0.20	0	82.76	None (see table 2)			
	0.10	0	85.58				
	0.05	0	82.69				

amount of chemical applied to each plant was the important criterion of successful control, and that no advantage resulted from increasing the volume of liquid above 63 ml. per plant. This fact, of course, is an important one in the application of this method on a large scale with mechanical transplanters, where the amount of liquid applied is usually 100 to 125 ml. per plant.

Commercial Application

Since the results already presented indicated that mercuric chloride was the most effective chemical, it was tried more extensively on a commercial

TABLE 4.—*The effect of various amounts of mercuric chloride, ethyl mercury chloride, and 2, 4, 5-trichlorophenol in the transplanting liquid upon the development of clubroot*

Chemical used	Amount of chemical applied per plant	Disease at end of season when the indicated amount of liquid per plant was used			Amount applied per acre	Cost per acre[a]
		63 ml.	125 ml.	250 ml.		
	Grams	*Index*	*Index*	*Index*	*Grams*	*Dollars*
None	.....	98.1	97.4	98.9	.....	.....
Mercuric chloride	0.218	20.0	30.7[b]	24.8[b]	1744	5.00
	0.088	38.6	31.7	32.9	704	2.00
	0.035	53.2	42.5	42.9	280	0.80
Ethyl mercury chloride (2% Ceresan)	0.475	88.5	64.7	58.9	3800	5.00
	0.190	74.8	77.4	89.1	1520	2.00
	0.075	83.3	72.9	85.7	600	0.80
2, 4, 5-trichlorophenol (Dowicide 2)	0.040	80.3	91.0	80.1	320	0.63
	0.015	90.1	92.9	81.4	120	0.25
	0.008	83.3	81.7	94.8	64	0.10

[a] Costs indicated are based on the rate of 8000 plants per acre and at the pre-war price of chemicals as follows: mercuric chloride, $2.87 per kilo; ethyl mercury chloride, $1.32 per kilo; 2, 4, 5-trichlorophenol, $1.98 per kilo.

[b] Difference in index required for significance (19: 1): between chemicals, 7.7; between amounts of chemical per plant or between amounts of liquid per plant within any one chemical, 13.3.

scale. In 1939 a trial on muck soil was conducted. Water was applied at the rate of 138 ml. per plant and there were approximately 8800 plants per acre. Mercuric chloride was added to make up the liquid at 1–1500 and 1–750 strengths. This amounted to 0.092 and 0.184 gram per plant. The results presented in table 5 show a marked reduction in disease index and increase of yield when the treated rows are compared with untreated. Although the index was lower and the yield higher with the 1–750 solution as compared with the 1–1500 treatment, the differences were not significant. In this instance, however, the increase in yield more than offset the increased cost of chemicals in the stronger treatment.

TABLE 5.—*The effect of mercuric chloride in the transplanting liquid upon clubroot development and yield in a commercial planting of cabbage on muck soil*

Concentration of mercuric chloride	Amount of liquid per acre	Amount of liquid per plant	Amount of mercuric chloride per plant	Amount of mercuric chloride per acre	Cost of mercuric chloride per acre[a]	Disease development[b]	Yield per acre[b]
	Gals.	*Ml.*	*Grams*	*Grams*	*Dollars*	*Index*	*Tons*
None	320	138	.....	.....	.....	85.3	7.82
1–1500	320	138	.092	810	2.32	36.7	12.51
1– 750	320	138	.184	1620	4.64	30.6	13.07
Difference required for significance (19: 1)	.....	.....	.....	.....	.....	11.3	2.02

[a] Cost is based on the prevailing wholesale price of mercuric chloride in 1939 of $2.87 per kilo.

[b] Disease development indices and yields are the means of 6 replicates.

Trials on a commercial scale were continued in 1940 and 1941. Four tests were made, 3 on muck and one on mineral soil. In one test mercurous chloride was compared with mercuric chloride. Disease indices, secured shortly before harvest, are given in table 6. Although mercurous chloride showed good promise in the exploratory tests (Table 2), it was definitely inferior to mercuric chloride in test 1 (Table 6). In all cases a decided reduction in disease index was secured with mercuric chloride. Concentrations of 1–750 and 1–1500 were, in general, the most satisfactory; usually, the differences between the two treatments, as in the commercial trial of 1940 (Table 5), probably were not significant. In tests 2, 3, and 4, HCl was used to facilitate dissolving the $HgCl_2$. No plant injury was noted on muck soil. In test 4 on mineral soil, however, there was some injury to plants in areas where the soil was comparatively dry at the time of transplanting.

TABLE 6.—*The effect of mercuric chloride and mercurous chloride in the transplanting liquid on clubroot development in commercial plantings*

Material added to transplanting liquid	Concentration of material in transplanting liquid[a]	Disease indices in tests indicated			
		Test 1, muck soil, 1940	Test 2, muck soil, 1941	Test 3, muck soil, 1941	Test 4, silt loam, 1941
None	.....	75.95	99.45	36.55	72.92
Mercuric chloride, $HgCl_2$	1– 375	.....	.....	.....	20.28
	1– 750	27.50	.....	16.70	24.10
	1–1500	48.08	58.51	21.95	15.91
	1–3000	51.42	.....	33.33	.....
Mercurous chloride, HgCl	1– 862	64.58	.....	.....	.....
	1–1725	63.46	.....	.....	.....
	1–3450	83.33	.....	.....	.....

[a] The liquid was applied at the approximate rate of 300 gal. per acre, and plants were set at about 8800 per acre.

DISCUSSION AND SUMMARY

The studies reported herein were conducted over a period of 4 seasons. They show conclusively that certain substantial benefits may be derived from the use of mercuric chloride in the transplanting liquid used for cabbage. The control accomplished is not complete but it is sufficiently effective to result often in increased yields many times the cost of treatment.

No encouraging results were secured with other inorganic or organic materials that were tested. For the time being it would appear that mercuric chloride alone is safe to recommend. It proved successful on both mineral and muck soils, and its effectiveness appears to be due to its fungicidal action in the soil zone around the tap root of the transplant. It is suggested that possibly mercury ionizes rapidly in the soil and permeates as a vapor for an appreciable distance. Mercury vapors arising from the soil have been shown to be toxic to certain potted plants in the greenhouse (28). This may account in part for the superiority of the mercury salts over nonmercury substances in this role.

The use of hydrochloric acid as a solvent is fraught with some danger of injury to the plants, especially on mineral soils. From the trials reported it would appear that the most effective treatments would be found within the range of 1–750 to 1–1500 applied at the rate of 60 to 125 ml. per plant.

There is nothing in the results reported to indicate that the control will be complete. Therefore, it should not be considered a substitute for such well-established practices as rotation, seed-bed sanitation, and, if feasible, maintenance of a neutral reaction by liming. As a supplement to these measures it should often be a worth-while procedure especially on mildly infested muck soils where the high buffer action often reduces or precludes any beneficial effect of liming in neutralizing the acidity of the soil solution.

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THE INFLUENCE OF GUTTATION FLUID ON PESTICIDES

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Since it was first discovered that Bordeaux mixture has fungicidal values, investigators have been perplexed by two problems involved in its use: (1) how does the insoluble copper in this mixture become soluble and therefore effective as a fungicide; and (2) how does this soluble copper at times enter and injure the leaf. The numerous publications of investigations within the past 60 years and the controversial nature of the reports are ample evidence of the complexity and importance of these problems.

There seems to be unanimity of opinion among investigators on several points: (1) copper, if effective as a fungicide, must be in a soluble or ionic form; (2) it does at times enter and injure the plant; (3) the copper in Bordeaux mixture is in an insoluble form; (4) some chemical action between the deposit and (a) the atmosphere, (b) meteoric water, (c) the fungus, or (d) the host plant or (e) other unknown agencies, to bring this insoluble copper into solution.

The object of this paper is to contribute certain new observations and experimental evidence that, when taken in consideration with the statements in the literature, may help to clarify these problems and may more fully explain the puzzling assortment of observations found in the literature concerning the physiological and chemical processes that are taking place between the pesticide and the host plant.

Since so many conflicting observations have been recorded concerning the capricious behavior of Bordeaux, only those references that may have a direct bearing on this investigation will be cited. Others (1, 2, 3, 6, 9, 10, 12, 13, 14, 17) have summarized quite adequately the observations and hypotheses previously advanced.

Barker and Gimingham (2) have grouped the various hypotheses concerning the solubility of copper in Bordeaux mixture, and its fungicidal value under these headings:

"1. The copper is brought into solution by atmospheric agencies—more especially by the action of the carbon dioxide of the air; *i.e.*, a purely chemical explanation.

"2. That the leaves, onto which the mixture is sprayed, exert a solvent action on the copper compounds, *i.e.*, an action of the host plant.

"3. That the fungus itself is responsible for the production of the soluble copper by which it is finaliy poisoned, *i.e.*, an action of the fungus."

McCallan (12, 13) and McCallan and Wilcoxon (14) attach little importance to CO_2 in the atmosphere or to uninjured leaves as having any solubilizing effect on copper in Bordeaux mixture. They do state, however, that meteoric water and injured or macerated leaves as well as fungous spores have the ability to release ionic copper in toxic concentrations from Bordeaux mixture.

TABLE 1.—*Chemical analysis of guttation fluid from plants*

Plant	Squash[a]	Tomato[b]	Tomato[c]	Squash[d]	Squash[e]	Cucumb's[f]	Cucumb's[g]	Squash[h]	Squash[i]	Cabbage[j]
Date	4–17–42	5–7–42	5–8–42	7–4–42	7–5–42	8–13–42	8–14–42	7–19–42	8–14–42	7–19–42
pH test	6.5	6.75	.....	7.30	7.10	6.00	6.48	6.30	7.30	6.08
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Chemical tests										
Nitrite nitrogen	.....	1.5	.....	.....	.....	1.0	1.0	.....	.....	.....
Nitrate nitrogen	250	1.0	1	1.0	10.0	3.0	1.0	5.0	13.0	4.0
Ammonia nitrogen	5	7.5	20	1.0	5.0	4.0	4.0	5.0	3.0	2.0
Phosphorus	75	2.0	5	2.0	1.0	8.0	8.0	1.0	1.5	4.0
Potassium	75	40.0	50	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Calcium	750	125.0	150	100.0	125.0	100.0	100.0	100.0	100.0	100.0
Magnesium	50	8.0	20	15.0	18.0	6.0	15.0	15.0	14.0	5.0
Aluminum	.....	0.3	.....	0.03	0.03	0.3	0.5	0.5	0.3	0.3
Manganese	.....	1.0	.....	T	T	T	.....	.....	T	1.0
Chlorine	.....	25.0	.....	.....	.....	50.0	25.0	25.0	25.0	35.0
Sulphate sulphur	.....	30.0	.....	.....	.....	25.0	25.0	25.0	25.0	25.0
Sodium	.....	T	.....	.....	.....	T	T	.....	.....	.....
Zinc	.....	.....	.....	5.0	5.0	.....	.....	.....	.....	.....
Copper	.....	.....	.....	.....	.....	.....	2.0	.....	.....	.....
Total solids	2500	600.0	.....	.....	.....	.....	.....	.....	.....	600.0
Organic	1100	275.0	.....	.....	.....	.....	.....	.....	.....	250.0

[a] Summer squash *Cucurbita pepo* growing in greenhouse 10 weeks old.
[b] Tomatoes growing in greenhouse 10 weeks old.
[c] Same as [b] except plants well fertilized with $(NH_4)_2SO_4$, treated with $(NH_4)_2SO_4$ on 5–6–42.
[d] Hubbard squash—Doolittle farm just before storm.
[e] Same as [d] except 1 day later.
[f] Cucumbers growing in greenhouse.
[g] Same as [f] except 1 day later.
[h] Summer squash—Mt. Carmel, Conn.
[i] Hubbard squash—same field as [d] and [e].
[j] Cabbage—Mt. Carmel, Conn.

It is with the second hypothesis that this paper is principally concerned. Wilson (18) pointed out that plants excrete fluid from uninjured leaves, which contains varying amounts of soluble materials. Table 1 gives the analysis of some of this fluid. This fluid or guttate, as is indicated in the table, was collected from a number of different plants both in the field and greenhouse and from the same plants on subsequent days. It was easily collected by sopping it up in glass wool and then squeezing it out into clean vessels. Chemical tests were made immediately and then the solution was quickly frozen to prevent any bacterial or mold action. These analyses are at best roughly quantitative and were made according to the method given by Morgan (15) for spot testing soil solution.[1] The total solids varied from approximately 2500 p.p.m. for No. 1 to approximately 600 p.p.m. for cabbage and tomatoes. Approximately 50 per cent of this material was of an organic nature.

The writer has advanced the hypothesis that the salts or compounds contained in the guttation fluid excreted from the host plant react or combine with the materials in sprays and dusts applied to plants as pesticides to form new compounds that either alter the effectiveness of the pesticide, or bring the copper into solution from the insoluble form from copper oxide and Bordeaux mixture. It has already been shown by Curtis (5) how guttation fluid is sucked back into the leaf. When this occurs, the soluble copper enters the plant to cause the well-known Bordeaux injury of Bordeaux-sensitive plants such as the cucurbits, or kill the leaf hopper (*Empoasca fabae* Herr) which causes hopper burn on potatoes.

It is a well-known chemical fact that one salt or compound has a very definite effect on the solubility of another. When plants that have been sprayed with Bordeaux mixture guttate, a whole series of inorganic salts in addition to unknown organic materials is brought directly in contact with the insoluble copper. An ideal condition is thus provided for the production of many new copper compounds. Tests were made to determine whether the guttation fluid could bring copper into solution from copper hydroxide. Ten ml. of distilled, tap, and guttation fluid from squash were agitated for 8 hours in flasks containing 100 mg. of copper hydroxide. When the supernatant solutions were filtered and tested,[2] no copper was found in either tap or distilled water, but 4 p.p.m. of copper were found in the solution containing guttation fluid. No copper was found in the guttation fluid as it came directly from the plant nor in either tap or distilled water.

INFLUENCE OF GUTTATION FLUID AND CERTAIN COMPOUNDS ON FUNGICIDAL EFFICIENCY

McCallan (12), Horsfall (11), and Dimond *et al.* (7), have described in detail methods for evaluating fungicides in the laboratory. A slight modi-

[1] The writer wishes to thank Miss Jane Andrews of the Soils Department of the Connecticut Agricultural Experiment Station for making these analyses.

[2] This test was made in the Department of Analytical Chemistry of the Connecticut Agricultural Experiment Station under the direction of Mr. W. T. Mathis.

fication of this method was used in determining the influence of guttation fluid on the toxicity of yellow copper oxide and Bordeaux mixture to *Macrosporium sarcinaeforme.*[3] This modification was: after the glass slides had been coated with the toxicants and dried, they were sprayed with two squeezes of a hand atomizer containing the guttation fluid and distilled water and held at constant level and distance from the slides. Table 2

TABLE 2.—*Effect of guttation fluid on copper toxicity*

Plants from which guttation water was collected	Spray time	Inhibition			
		Yellow copper oxide		Bordeaux mixture	
		Over sprayed with			
		Distilled water	Guttation fluid	Distilled water	Guttation fluid
	Seconds	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Summer squash growing in greenhouse, collected April 17, 1942, spore-tested April 17, 1942	40	100	100	90	100
	20	65	100	99	100
	10	46	100	89	100
	5	3	91	4	99
Tomatoes growing in the greenhouse, 12 weeks old, collected June 15, 1942, spore-tested Oct. 6, 1942	40	75	99	99	100
	20	51	94	91	99
	10	41	83	46	65
	5	28	66	8	23
Tomatoes in greenhouse, 10 weeks old, collected May 12, 1942, spore-tested May 12, 1942	40	.....	100	81	99
	20	47	93	50	95
	10	19	95	22	65
	5	11	28	10	7
Same as above except that the plants were fertilized with NH_4SO_4 on May 6, 1942	40	.....	91	81	89
	20	47	71	50	80
	10	19	12	22	9
	5	11	6	10	2
Cucumbers growing in the greenhouse, collected Aug. 13, 1942, spore-tested Oct. 6, 1942	40	73	100	99	99
	20	51	99	91	98
	10	41	95	46	79
	5	28	86	8	19
Same plants as above except guttate was collected Aug. 13, 1942	40	73	92	99	99
	20	51	96	91	98
	10	41	85	46	88
	5	28	70	8	41
Cabbage growing at the Exp. Station farm, Mt. Carmel, collected July 19, 1942, spore-tested Oct 6, 1942	40	73	100	99	100
	20	51	84	91	98
	10	41	59	46	59
	5	28	44	8	19
Summer squash growing at the Exp. Station farm, Mt. Carmel, collected July 19, 1942, spore-tested Oct. 6, 1942	40	73	88	99	99
	20	51	75	91	93
	10	41	66	46	67
	5	28	31	8	21

and figure 1[4] give the results of these tests, which show clearly that the guttation fluid increased to a marked degree the per cent of inhibition of spore germination. In figure 1 not only are all the lines from the guttation fluid series parallel to each other, but their slope is also different from the

[3] Thanks are given to Dr. James G. Horsfall, Dr. Albert Dimond and Miss Florence McWilliam of the Department of Botany and Plant Pathology of the Connecticut Agricultural Experiment Station for making these spore tests.

[4] The advantages of plotting these data on Logarithmetic Probability paper and the L.D. 50 values referred to are discussed in detail by Dimond *et al.* (7).

distilled water check. This difference in slope shifts the L.D. 50 values decidedly to the left, thus indicating that the guttation fluid brought more copper into solution to inhibit spore germination than did distilled water. These results support the hypothesis presented by the writer in the preced-

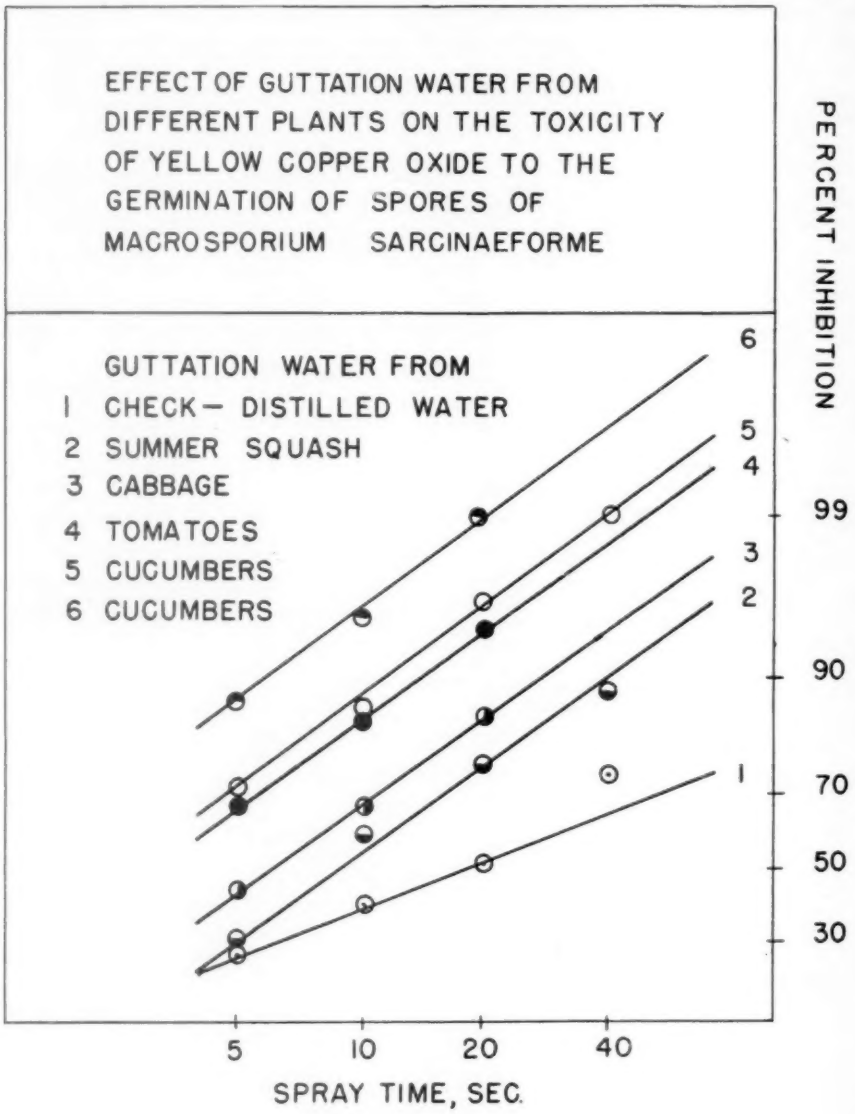

FIG. 1

ing paragraphs. Furthermore, the parallel lines in figure 1 indicate that the guttation fluid from different species of plants affect the copper oxide in the same way but at different rates. This fact presents the question: is there some one compound common to the guttation fluid from all plants, but

present in different amounts? Since ammonia readily forms complex compounds with copper, perhaps the ammonia was the most likely compound to give this effect. The results of a preliminary experiment in which 20, 10, and 5 p.p.m. of ammonium chloride and ammonium hydroxide tested in the same way as the guttation fluid, are given in table 3. The ammonium

TABLE 3.—*Effect of NH_4CL and NH_4OH on copper toxicity*

Chem. compound and p.p.m.	Spray time	Inhibition			
		Yellow copper oxide		Bordeaux mixture	
		Over sprayed with			
		Distilled water	Salt solution	Distilled water	Salt solution
	Seconds	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ammonium chloride					
20 p.p.m.	40	95	100	98	100
	20	85	100	86	94
	10	52	97	33	61
	5	10	60	9	12
10 p.p.m.	40	95	100	98	95
	20	85	99	86	95
	10	52	88	33	47
	5	10	38	9	12
5 p.p.m.	40	95	100	98	98
	20	85	97	86	93
	10	52	93	33	56
	5	10	62	9	11
Ammonium hydroxide					
20 p.p.m.	40	94	99	.....	.....
	20	82	91	.....	.....
	10	51	61	.....	.....
	5	12	16	.....	.....
10 p.p.m.	40	94	96	.....	.....
	20	83	94	.....	.....
	10	51	37	.....	.....
	5	12	19	.....	.....
5 p.p.m.	40	94	96	.....	.....
	20	82	85	.....	.....
	10	51	40	.....	.....
	5	12	10	.....	.....

chloride increased the per cent of inhibition of spore germination above the distilled water check on both yellow copper oxide and Bordeaux. While the difference between the ammonium hydroxide and distilled water on yellow copper oxide is less pronounced, there is, nevertheless, an increased inhibition.

A second experiment was made in which 6 salts, ammonium citrate, ammonium chloride, ammonium sulphate, sodium citrate, potassium chloride, and potassium sulphate were used in concentrations of 20 and 10 p.p.m. The results (Table 4) show that all the salts increased the per cent of inhibition of spore germination over that of distilled water. On the yellow copper oxide the ammonium salts had a greater effect than either sodium or potassium, while there was little difference on the Bordeaux series between any of the salts.

TABLE 4.—*Effect of different salts on copper toxicity*

Chem. compound and p.p.m.	Spray time	Inhibition			
		Yellow copper oxide		Bordeaux mixture	
		Over sprayed with			
		Distilled water	Salt solution	Distilled water	Salt solution
	Seconds	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ammonium citrate 20 p.p.m.	40	19	53	90	99
	20	13	31	78	82
	10	5	11	16	29
	5	5	7	6	8
10 p.p.m.	40	19	70	90	99
	20	13	18	78	82
	10	5	7	16	16
	5	5	4	6	5
Ammonium chloride 20 p.p.m.	40	19	93	90	97
	20	13	74	78	62
	10	5	21	16	31
	5	5	8	6	8
10 p.p.m.	40	19	62	90	98
	20	13	34	78	78
	10	5	14	16	30
	5	5	6	6	9
Ammonium sulphate 20 p.p.m.	40	19	67	90	99
	20	13	12	78	87
	10	5	6	16	19
	5	5	2	6	12
10 p.p.m.	40	19	50	90	92
	20	13	13	78	69
	10	5	4	16	11
	5	5	1	6	3
Sodium citrate 20 p.p.m.	40	19	35	90	97
	20	13	15	78	86
	10	5	7	16	21
	5	5	5	6	5
10 p.p.m.	40	19	23	90	98
	20	13	13	78	70
	10	5	7	16	34
	5	5	2	6	6
Potassium chloride 20 p.p.m.	40	19	33	90	99
	20	13	8	78	77
	10	5	5	16	16
	5	5	1	6	4
10 p.p.m.	40	19	44	90	.....
	20	13	8	78	66
	10	5	5	16	24
	5	5	1	6	4
Potassium sulfate 20 p.p.m.	40	19	43	90	94
	20	13	10	78	67
	10	5	4	16	33
	5	5	5	6	8
10 p.p.m.	40	19	22	90	88
	20	13	11	78	64
	10	5	4	16	35
	5	5	1	6	5

Baine (1) lists $CaCl_2$, $Ca(NO_3)_2$, NaCl, and $Mg(NO_3)_2$ as having materially increased Bordeaux injury to peach leaves while KNO_3, cane and grape sugar were less effective, $Ca_3(PO_4)_2$, K_3PO_4 were indifferent, and KH_2PO_4 seemed to diminish the injury. It would be difficult to determine which or how much of the compounds Baine (1) used in this experiment, or those of the writer, occur in the guttation fluid. From the results of these experiments, however, there can be little doubt that the compounds in solution in the guttation fluid must at times play an important part in bringing copper into solution from the insoluble form to increase its effectiveness as a fungicide, and that the ammonia in this fluid must be an important constituent in bringing about this effect. Other compounds, either singly or collectively, undoubtedly have similar effects.

Crandall (4) was probably, at least in part, correct in his statement about the ammonia contained in the rain having a solubilizing effect on the copper fungicide. Failyer and Willard (8) have shown that rain water, especially in amounts of less than 2.5 millimeters, contains appreciable amounts of ammonia. In one such rain they reported 12.2 p.p.m. of nitrogen. According to the experiments described above this would certainly be sufficient amount to bring copper into solution from Bordeaux.

THE RELATION BETWEEN GUTTATION FLUID AND SPRAY INJURY

A good many observations have been made by different investigators as well as fruit and vegetable growers concerning the injury to certain plants following the use of Bordeaux mixture or other sprays or dusts. There seems to be an unanimity of opinion among them that this injury is nearly always associated with wet, foggy weather or conditions of high temperature and humidity. Frequent reference, Baine (1), Crandall (4), also is made to heavy dews and meteoric waters on the sprayed foliage, which later shows injury. Furthermore, they are universally agreed that the copper must enter the leaf to cause injury, yet its method of entry is still a controversial subject. DeLong (6) has given an excellent review of most of the literature on this subject.

In only one of these observations, as far as the writer has been able to determine, has guttation fluid been collected and its solubilizing effect determined on a spray or dust. Smith (16) collected the "dew" from cotton plants and found that it increased markedly the solubility of calcium arsenate. He attributed this effect to the chemical action of the compounds in the dew on the calcium arsenate. Unquestionably, Smith, in this work, found the most important factor that was responsible for the increased solubility of calcium arsenate when it was applied to cotton as an insecticide. He concluded that the soluble arsenate is responsible for the injury of this dust to the plant. Although the title of this paper is "Excretions from Leaves as a Factor in Arsenical Injury to Plants," he was uncertain how these compounds got into the dew. He says: "Whether these have come into the dew by osmosis or by actual exudation was not determined, the for-

mer being more probable." In discussing the influence of various chemical compounds, found as impurities in the insecticide or in the water with which spray and dusts are mixed, on the solubility of arsenicals he makes this statement: "Their work suggests that the burning of di-lead arsenate frequently observed on the Pacific Coast, and hitherto attributed to hydrolysis by the heavy and recurring fogs of that region, is really due to the salt spray entrapped by these fogs." It seems to the writer that there was little need of looking for an outside agency in supplying chemical impurities when, under foggy conditions the plant probably would guttating to supply them.

Baine (1) made some pertinent statements concerning the entrance of copper into the peach leaf. In every instance in which he refers to this he emphasized the necessity of moisture being present on the leaf. ". . . it may be true that there is sufficient moisture in the cuticle of the living leaf to allow for the diffusion of a minute quantity of copper into the leaf." In another paragraph in which he discusses his experiments with deliquescent copper salts on peach leaves he states, "Here the salts absorb moisture from the rapidly cooling air in the afternoon and become a saturated solution just at the most favorable time for its entrance into the leaf by imbibition." He was not sure whether the copper entered by diffusion through the cuticle or by imbibition. Where deliquescent copper salts were used he was certain that it entered by imbibition. This action he described as occurring especially toward evening when the dew begins to fall during showery weather.

In the writer's opinion Baine, Smith, and Crandall all observed the solubilizing influence of guttation water on Bordeaux mixture, and much of the meteoric water and dew which they discuss was actually fluid excreted or guttated by the plant.

It is possible that Baine actually saw the leaf imbibe water in the same way that the writer, Curtis (5), has seen and demonstrated that the guttation fluid is sucked back into the leaf. The writer is certain, however, that Baine did not see dew "fall" in the evening.

SUMMARY

Chemical analyses of guttation fluid collected from tomatoes, cabbage, cucumbers, summer and Hubbard squash showed that the water contains in solution both organic and inorganic salts of varying concentrations and pH, that the solutes from the same plants may vary from day to day, and that the concentration of the solutes may be influenced by soil fertilization.

The guttation fluid from squash plants was shown to bring copper into solution from $Cu(OH)_2$; furthermore, the guttation fluid from all the plants tested increased the toxicity of yellow copper oxide and Bordeaux mixture when sprayed on glass slides and seeded with known dilutions of *Macrosporium sarcinaeforme*. Different concentrations of several salts gave similar effects.

The hypothesis advanced to explain how sprays and dusts applied as pesticides injure the plants is that the guttation fluid increases the solubility of the pesticide, which is then sucked back into the leaf in the guttation drops so that injury occurs from the inside of the leaf. This hypothesis can explain also the process by which on plants previously sprayed with Bordeaux the copper content of potato leaves can be built up in concentrations toxic to the potato leaf hopper, *Empoasca fabae* (Herr).

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THE PERFECT STAGE OF COLLETOTRICHUM FALCATUM

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The fungus causing the red rot of sugar cane, *Colletotrichum falcatum,* was first described from Java by Went in 1893 (6). In the imperfect stage, the only stage known until 1942, falcate, unicellular, hyaline conidia develop in acervuli on conidiophores, which are intermixed with numerous setae. The conidia form on short conidiophores and, sometimes, even on the setae. The conidia upon germination may either develop a mycelium directly or produce appressoria similar to those produced by certain other members of the order Melanconiales. The fungus fruits on various parts of the sugar-cane plant, but the acervuli are particularly common on the midribs of the leaves. The fungus occurs in all sugar-producing countries, and, in some, causes serious losses to the cane industry. In Louisiana it has been known since 1908 (3, 4) and is recognized as one of the most serious and important disease-producing organisms on sugar cane.

In 1942, while studying the red-rot fungus on some cane leaves that had been sterilized in the autoclave and inoculated with a pure culture, a perithecial stage, later identified as belonging to the genus *Physalospora,* was observed. This ascigeral stage has since been found very commonly prevalent in the field under certain conditions, and has been produced in the laboratory on cane leaves, either sterilized or not, and on other fibrous material. It has been proved to be the perfect or ascigeral stage of *Colletotrichum falcatum.*

OCCURRENCE OF PERITHECIA ON CANE LEAVES

In the field the perithecia have been seen most commonly on dead leaf blades and leaf sheaths that were beginning to dry. Leaves on shoots killed by too much crowding or from other causes frequently have been found covered with perithecia. It also has been possible usually to hasten perithecial formation by cutting leaves showing midrib lesions and placing them on moist cotton in moist chambers. Generally, as the tissues have lost their chlorophyll and died, the perithecia have developed and matured. The perithecia have occurred in abundance on the leaf sheaths, on the underside of the midrib, and on both surfaces of the rest of the leaf blade.

The perithecia generally have been found under field conditions somewhat later than the period of greatest conidial production; and this, combined with the fact that they have not been very conspicuous, is apparently the reason why they have not been reported previously.

Perithecia have been collected in Louisiana on plants of the 5 recognized species of *Saccharum, S. officinarum* L., *S. barberi* Jeswiet, *S. sinense* Roxb., *S. spontaneum* L., and *S. robustum.* They also have been collected on leaves of the grass *Leptochloa filiformis* (Lam.) Beauv., which is very common in

sugar-cane fields in Louisiana. They also have been collected on numerous hybrids of the above cane species. There is no reason to doubt their development on all cane varieties.

In the laboratory, perithecia have developed not only on diseased cane leaves brought in from the field, but also upon sterilized leaves of cane, sorghum, and corn placed on wet cotton in Petri dishes, and even on sterilized strips of filter paper placed on cotton wet with a nutrient solution in Petri dishes following inoculation with a pure culture of the red-rot fungus.

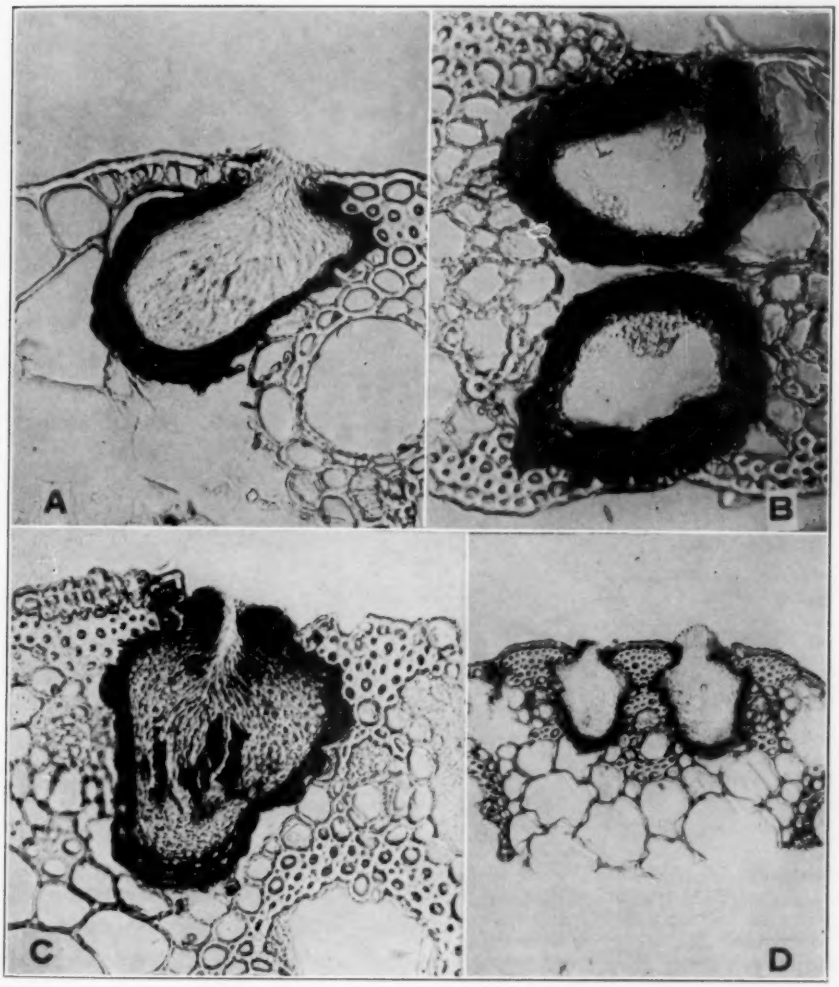

FIG. 1. Perithecia of *Physalospora tucumanensis*. A. Perithecium from upper surface of leaf. ×240. B. Perithecia on both surfaces of leaf. ×240. C. Perithecium showing usual position between fibrovascular bundles. ×240. D. Two perithecia closely associated with fibrovascular bundles. ×120.

STRUCTURE OF PERITHECIA

On a leaf the perithecia normally develop between fibrovascular bundles, filling all the available space (Fig. 1). In a cross section of a leaf the peri-

thecia may appear to be quite irregular due to the perithecial wall following the contours of the bundles. In a longitudinal direction, with no restricting bundles, the perithecia are usually considerably wider and the walls are rounded (Fig. 2, D). The perithecia are almost entirely under the surface with only a very small portion of the ostiole outside the epidermis. At maturity the perithecia are filled with a large number of asci and numerous paraphyses (Fig. 3). The paraphyses, which extend to the ostiole, are deli-

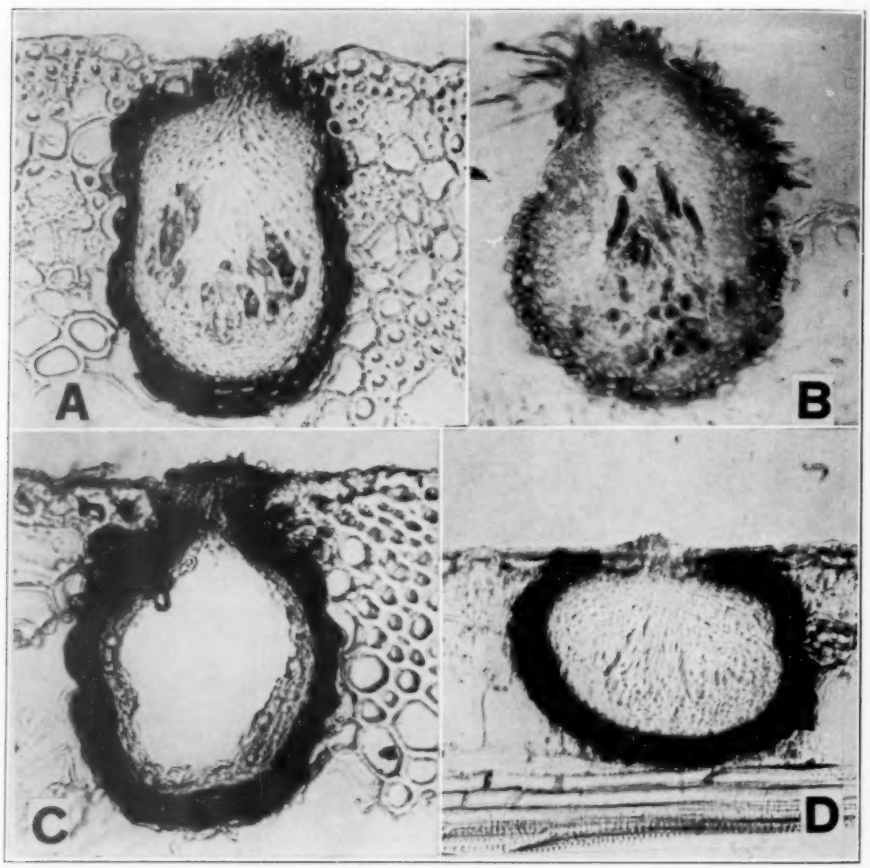

FIG. 2. Perithecia of *Physalospora tucumanensis*. A. Perithecium with asci. ×300. B. Perithecium from autoclave-sterilized leaf inoculated with red-rot culture. ×240. C. Perithecium from type material from Argentina. ×300. D. Young perithecium, section cut parallel to long axis of leaf. ×240.

cate structures. In a smear preparation, their walls are difficult to see, but the abundant granules or oil drops in the interior are very conspicuous. The asci are club-shape and somewhat thickened at the apices. The ascospores are elliptical, hyaline, unicellular, straight or slightly fusoid, each containing, like the conidia of *Colletotrichum falcatum*, a central clear area, which is assumed to be a nucleus.

Perithecia that develop on sterilized leaves are larger, more thin-walled, and irregular, and project farther from the surface than those on leaves from the field (Fig. 2, B).

ISOLATION AND INOCULATION TESTS

To study the *Physalospora* from sugar cane, and to prove beyond question its relation to the red-rot fungus, cultures were obtained from ascospores from various cane varieties and from all the cane species and the grass *Leptochloa filiformis*. In the course of the study, at least 497 pure cultures were obtained from single ascospores from perithecia that developed naturally on leaves. These were picked up with the micromanipulator, germi-

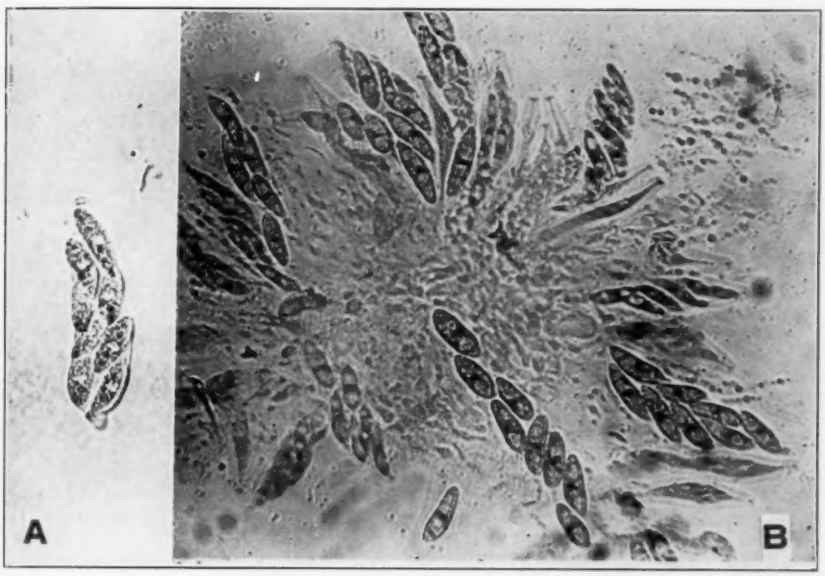

FIG. 3. Asci of *Physalospora tucumanensis*. A. Single ascus, not stained, showing light-colored area in center of each spore. ×400. B. Mass of asci from a perithecium, stained with Cotton Blue. ×400.

nated, and transferred to culture tubes. These cultures grew rapidly and produced in abundance falcate conidia typical of *Colletotrichum falcatum*. In no way did these cultures differ from those obtained from conidia of the red-rot fungus. Cultures of both the light and dark strains, as described by Abbott (1), were obtained from single ascospores. Of the 497 cultures studied, 357 were of the dark strain or similar to it, while 140 were of the light strain. In some cases both strains were obtained from the same midrib or leaf sheath material, though in no case did both strains come from the same perithecium.

To prove with certainty that the *Physalospora* found on sugar cane was the perfect stage of the red-rot fungus, *Colletotrichum falcatum*, numerous inoculation experiments were made on sugar cane with conidia that de-

veloped in single ascospore cultures. In all, more than 50 different cultures were used in the tests. Both leaves and stalks were inoculated.

Leaves in the field were inoculated by inserting conidia in punctures on the upper surface of the midrib. Three leaf inoculation tests were carried on at various times during the summer, using a number of different ascospore cultures along with known red-rot cultures. The lesions produced on the inoculated leaves were in all ways similar to lesions produced by virulent red-rot cultures. They were elongated, reddish to brownish, usually with a

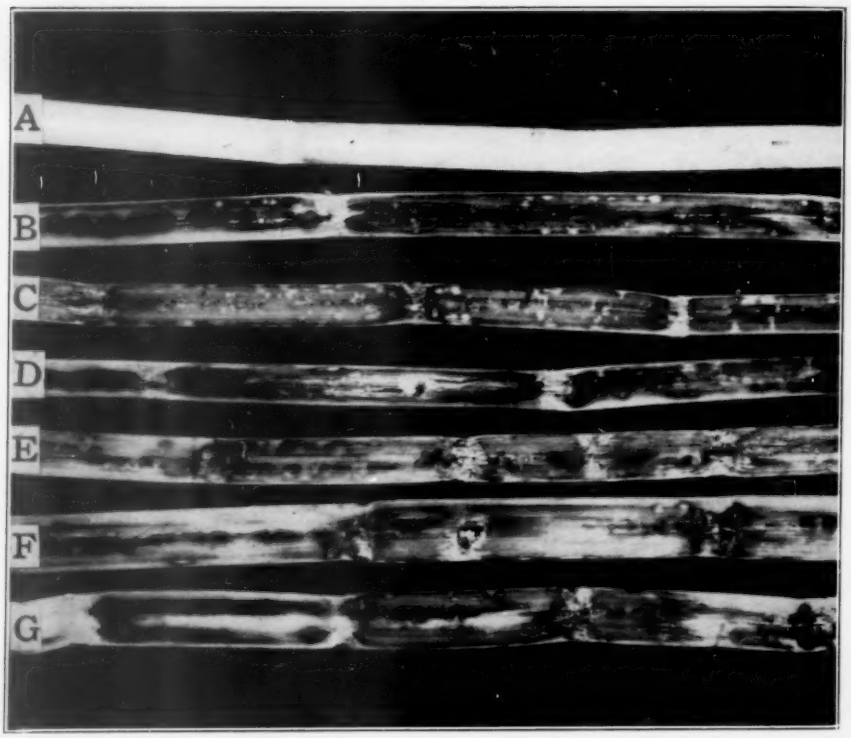

FIG. 4. Red rot in stalks of cane following inoculation with single-spore cultures of *Colletotrichum falcatum* and *Physalospora tucumanensis*. A. Control, not inoculated. B. Co. 281 inoculated with single-ascospore culture. C. Co. 281 inoculated with single-conidium culture of *Colletotrichum falcatum*. D. Co. 281 inoculated with single-ascospore culture isolated from a leaf that had been inoculated with a culture of *Colletotrichum falcatum* that had been carried through 40 generations by conidial transfers. E. Co. 281 inoculated with single-ascospore culture. F, G. C.P. 33/243 inoculated with diffierent single-ascospore cultures.

light-colored central area on which the acervuli developed in abundance. Many of the inoculated leaves showing lesions were cut and placed in moist chambers. As the green color faded after several days, perithecia developed in abundance on them.

The inoculations in the stalks were of 3 types, these being similar to the routine tests made in testing seedlings and new canes for resistance to red rot. These included inoculations in standing cane in the field, inoculations

in cut stalks kept in the laboratory, and inoculations in cut stalks just previous to planting in the field. All stalk inoculations were made by inserting spores in the stalks in holes made with a metal inoculator, a cutting instrument that removes a plug of stalk tissues.

Inoculations in the field were made at various times using single ascospore cultures along with known virulent red-rot cultures. In such tests the conidia migrated up and down in the fibrovascular bundles. Red lesions developed along the bundles wherever the spores lodged and germinated.

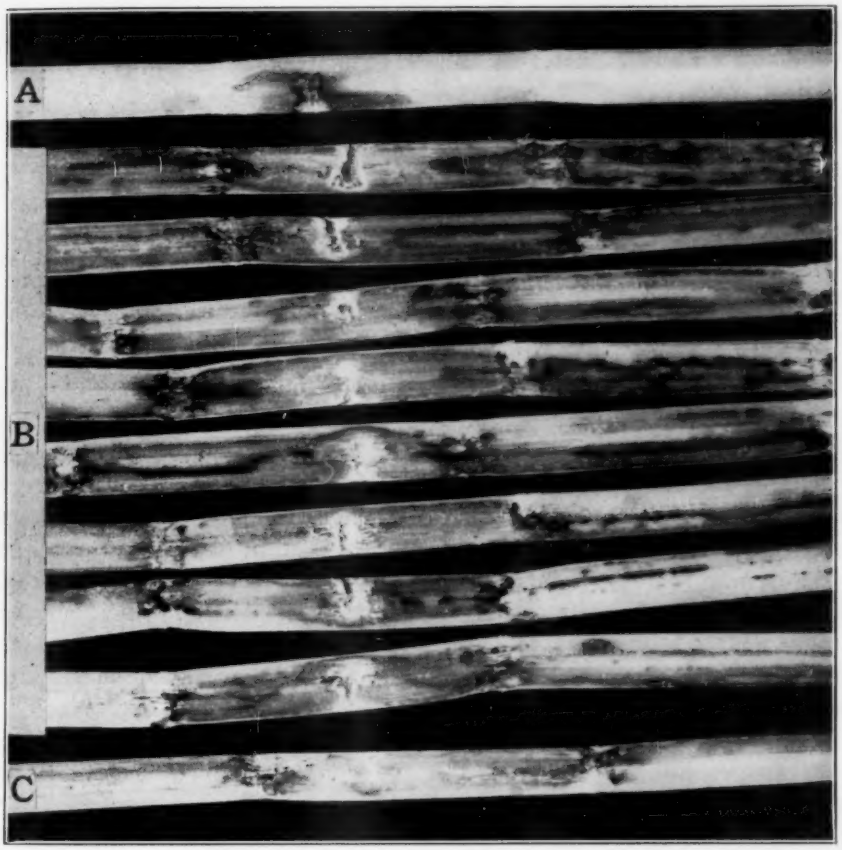

FIG. 5. Test with cultures from ascospores from a single ascus. A. Control, not inoculated. B. Stalks inoculated with cultures from 8 ascospores from one ascus. C. Stalk inoculated with single-ascospore culture isolated from a leaf that had been inoculated with a culture of *Colletotrichum falcatum* that had been carried through 40 generations by conidial transfers.

Many ascospore cultures were tested and all produced the same typical red lesions, as did the red-rot cultures.

Inoculations with many cultures also were made in cut stalks stored in the laboratory. These all produced the more generalized type of injury characteristic of stalks in this condition.

In stalks planted in the fall after inoculation, the mycelium spread through the stalks during the fall and winter months. The rot produced by

ascospore cultures was identical with that produced by virulent red-rot cultures (Fig. 4).

Numerous cultures were made from the inoculated leaves and stalks and in all cases typical red-rot cultures were obtained.

HOMOTHALLISM OF PHYSALOSPORA

The inoculation tests and culture experiments also have demonstrated that the sugar-cane *Physalospora* is homothallic. It always has been possible to force the formation of perithecia on leaves or leaf sheaths inoculated with single ascospore cultures. Also, perithecia have been produced in abundance on sterilized leaves and other materials inoculated with such cultures.

RESULTS WITH ASCOSPORES FROM SAME ASCUS

In several of the experiments with the sugar-cane *Physalospora*, it was possible to obtain pure cultures from all 8 ascospores from a single ascus. In all cases these cultures were absolutely identical as far as could be determined. Furthermore, these were used in inoculation tests, and the same symptoms were produced by all cultures (Fig. 5).

DETERMINATION OF LOUISIANA FUNGUS

In the attempts to determine the *Physalospora* on sugar cane in Louisiana, its characters were compared with those of similar fungi reported on sugar cane and related plants in various parts of the world. Of the several species of *Physalospora* reported on sugar cane, the characters of one, *P. tucumanensis* Speg., seemed to agree closely with those of the Louisiana material. This fungus was first described in 1896 in Argentina by Spegazzini (5), who found it occurring commonly on dying leaves of sugar cane. In order to determine with reasonable certainty whether or not the Louisiana fungus was the same as that from Argentina, a fragment of the type material of Spegazzini's fungus, *Physalospora tucumanensis*, was obtained from Argentina.[1]

This fragment contained perithecia (Fig. 2, C) in abundance and also setae and conidia of what seemed to be *Colletotrichum falcatum*. Upon examination, it was found that the perithecia, asci, and ascospores were very similar to those of the fungus found in Louisiana, which had been proved to be the perfect stage of *Colletotrichum falcatum*. With this evidence, the Louisiana fungus was reported as *Physalospora tucumanensis* Speg. (2).

DESCRIPTION OF P. TUCUMANENSIS

The characteristics of *Physalospora tucumanensis* based on studies of Louisiana collections are given in the following description.

Physalospora tucumanensis Speg. Perithecia mostly scattered, sometimes 2 or 3 together, on various parts of host plant but abundant on leaf sheath and leaf blade, nearly submerged with only a small portion of ostiole protruding, located usually between fibro-

[1] Type material was kindly furnished by Juan C. Lindquist of the Instituto de Botanica "Spagazzini," La Plata, and was labeled "No. 418, separado del tipo."

vascular bundles and often filling entirely the space between the bundles, black, 100–260 (width) × 85–250 (height) μ in size, the width in longtudinal section of leaf greater than in cross section. Asci numerous, clavate, thickened at the apex, 50–118 × 7.4–19.2 μ, usually ranging from 70–90 × 13–18 μ. Ascospores irregularly biseriate, single-celled, hyaline, straight or somewhat fusoid, elliptical to ovate, at maturity and, while fresh, containing a hyaline area near center, 12.5–30.0 × 5.0–11.1 μ, usually ranging from 18–22 × 7–8 μ. Paraphyses very abundant, delicate walls, septate, usually unbranched, filled with conspicuous granules or oil droplets, extending to ostiole. Periphyses abundant and conspicuous around ostiole opening.

Found in abundance on dead and dying leaves of species of *Saccharum* and *Leptochloa filiformis* in Louisiana, usually following conidial formation of *Colletotrichum falcatum*. Proved to be the ascigeral stage of *Colletotrichum falcatum* Went.

SUMMARY

A fungus belonging to the genus *Physalospora* has been found in abundance on dead and dying leaves and other parts of species of *Saccharum* and *Leptochloa filiformis* in Louisiana usually following the production of conidia of *Colletotrichum falcatum*.

By inoculation tests, the *Physalospora* has been proved to be the ascigeral stage of *Colletotrichum falcatum*, the fungus causing the red rot of sugar cane.

The perithecia have been produced in the laboratory on sterilized leaves and other fibrous material.

The *Physalospora* is homothallic, as perithecia have been readily produced with single ascospore cultures.

The 8 ascospores from an ascus have produced identical cultures with the same pathogenicity.

The fungus has been identified as *Physalospora tucumanensis* Speg., a species collected in Argentina by Spegazzini and described by him in 1896. The identification has been confirmed by comparing the Louisiana material with type specimens of *P. tucumanensis*.

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FURTHER STUDIES ON A SPECIES OF HELMINTHOSPORIUM PARASITIZING CORN[1]

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INTRODUCTION

In 1941 (6) a species of *Helminthosporium* was reported occurring as a parasite on dent corn (*Zea mays* L.) in the Corn Belt. The species was shown to be composed of two physiological races, morphologically indistinguishable, but separable on the basis of symptoms each produced and the high degree of specialization in parasitism of one of the races toward certain inbred lines of corn. Susceptibility to this race was shown to be inherited as a monogenic recessive (7).

Morphological comparisons between the species reported and that of *Cochliobolus heterostrophus* Drechs. (*Helminthosporium maydis* Nisikado and Miyake) as found in the literature (1, 2, 3, 4), indicated close agreement in range of spore length, range of spore width, number of septations, character of the hilum, mode of spore germination, and range in length of conidiophores. Symptoms on mature leaves also bore similarity to those caused by *C. heterostrophus*, as shown in illustrations (2), and as seen on examination of herbarium material. The fungus under consideration was identified as *H. maydis* because of these similarities in morphology and in symptoms it produced.

At the time the study was being made, attempts to obtain viable collections of *Helminthosporium maydis* from the Southern States for comparison were unsuccessful. In the late summer of 1942, a relatively severe epidemic of leaf spot of corn caused by *H. maydis* occurred in the Southeastern States (8). Through the courtesy of George Y. Young several collections of *H. maydis* were obtained for comparison with the species occurring in the Corn Belt states.

OBSERVATIONS

Pure cultures of *Helminthosporium maydis* were obtained from 4 collections made in Mississippi and from 1 collection made in North Carolina. At the same time pure cultures were made from collections of the species occurring in the Corn Belt. Of the latter cultures, 3 were of race I and 3 of race II. Spore suspensions of each were sprayed on seedlings of inbred lines, single crosses, and double crosses. The seedlings were placed in moist chambers overnight and then returned to the greenhouse bench. Care was taken to prevent any mixing of inoculum before and after spraying on the

[1] Cooperative Investigation, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station. Journal Paper No. 116 of the Purdue University Agricultural Experiment Station.

[2] Associate Pathologist, Division of Cereal Crops and Diseases.

plants. One week after inoculation, when symptoms were well established, the results were recorded as shown in table 1. These data indicate no specialization in parasitism among the southern isolates for the inbred lines and hybrids inoculated. Tests were continued to include 57 inbred lines, 8 single crosses, and 6 double crosses. All material tested showed equal susceptibility to all isolates from southern collections.

The symptoms produced by races I and II on seedling leaves (Fig. 1, A), as well as on mature plant leaves, are distinct from each other and have already been described (6). The symptoms produced by the southern iso-

TABLE 1.—*Reaction of inbred lines, single crosses, and double crosses to inoculation with isolates of Helminthosporium sp. from Corn Belt collections, and with isolates of H. maydis collected in the Southern States*

Inbred or hybrid inoculated	Isolates used for inoculation										
	Isolates of the Corn Belt species						Isolates of *H. maydis*[d]				
	Race I			Race II			Mississippi				N.C.
	(K61)[a]	(K44)	(Pr)	(P8)	(187–2)	(WF9)	No. 1	No. 2	No. 3	No. 4	No. 1
Inbreds											
K61	S[c]	S	S	M.S.	M.S.	M.S.	S	S	S	S	S
K44	S	S	S	"	"	"	"	"	"	"	"
Pr	S	S	S	"	"	"	"	"	"	"	"
Pr 1[b]	R	R	R	"	"	"	"	"	"	"	"
187–2	R	R	R	"	S	"	"	"	"	"	"
38–11	R	R	R	M.R.	M.R.	M.R.	"	"	"	"	"
Single crosses											
K61 × Pr	S	S	S	M.S.	M.S.	M.S.	"	"	"	"	"
K61 × K44	S	S	S	"	"	"	"	"	"	"	"
187–2 × L317	R	R	R	"	"	"	"	"	"	"	"
Double crosses											
U. S. 13	R	R	R	"	"	"	"	"	"	"	"
Ind. 844	R	R	R	"	"	"	"	"	"	"	"

[a] Designations in parentheses are of inbred lines from which isolates were obtained.
[b] Homozygous resistant inbred Pr obtained by outcrossing to a resistant inbred, backcrossing five successive generations, and selfing twice.
[c] S. = Susceptible; R. = Resistant; M.S. = Moderately susceptible; M.R. = Moderately resistant.
[d] From the Southern States.

lates on seedling leaves, while resembling those caused by race I, are nevertheless distinct (Fig. 1, B). In the early stages of development of the lesions, water soaking of the tissues is a conspicuous symptom. Young lesions produced by race I, while they do show water soaking, do not do so to the extent found in infections caused by *H. maydis* from southern collections. As compared with symptoms of race I, fully developed lesions on seedling leaves caused by the southern isolates are somewhat more elongated and the margins of the lesions are more irregular. A definite zonate pattern is characteristic of the lesions of both diseases. In those infections caused by the southern isolates a purplish-brown margin surrounding the dry straw-colored area is a distinct feature.

Morphological Comparisons

Spore suspensions of the southern isolates and of race I and of race II of the Corn Belt species were separately sprayed on seedlings of K61 × Pr, a single cross susceptible to all cultures. Ten days after inoculation individual collections of seedling leaves, each of which was infected with a single

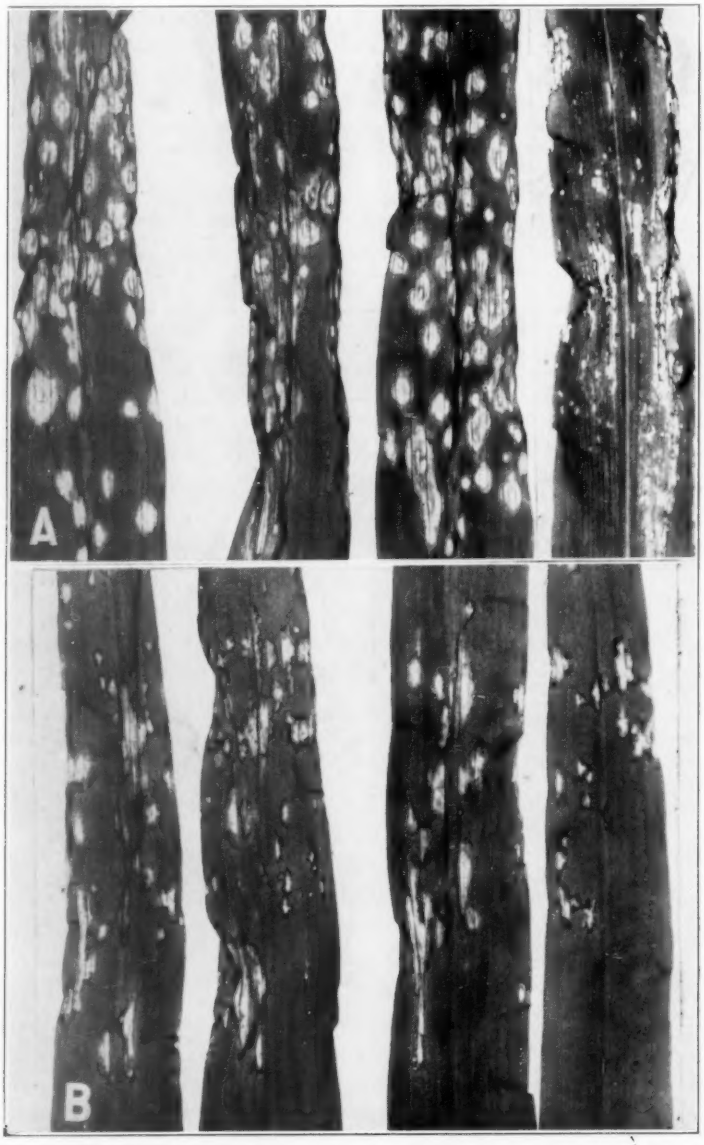

FIG. 1. A. Symptoms on seedling leaves of K61 × Pr, produced by isolates of *Helminthosporium carbonum*. The first three leaves infected with isolates of race I, the fourth leaf infected with an isolate of race II. B. Symptoms on seedling leaves of K61 × Pr produced by isolates of *H. maydis* collected in the Southern States.

culture, were harvested and dried at room temperatures for 2 weeks. The dried leaves were then placed in moist chambers for 4 days at which time spores had been produced in abundance on the lesions. Spores were scraped at random from lesions scattered over the leaves and spore length, spore width, and number of septations recorded. Three samplings totaling 105 spores were made from each culture. The data obtained are shown in table 2. Data for spore length of the southern collections are compared with

TABLE 2.—*Comparative measurements of spores grown from collections of Helminthosporium maydis and H. carbonum on seedling leaves of the susceptible single cross K61 × Pr*

Culture No.	Range of spore length	Mean spore length	Range of spore width	Mean spore width	Range in number of septa	Mean number of septa
			Southern collections (*H. maydis*)			
	Microns	*Microns*	*Microns*	*Microns*		
1	42.5–127.4	86.7	10.6–21.2	15.2	5 – 11	8.1
2	35.4–120.4	92.9	7.1–17.7	15.7	3 – 13	8.5
3	24.8–120.4	91.8	10.6–17.7	14.3	3 – 12	8.5
4	31.9–123.9	85.5	10.6–17.7	14.4	3 – 11	8.2
5	46.0–127.4	89.1	10.6–17.7	14.6	3 – 12	8.2
Grand means		89.2		14.9		8.3
			Northern collections (*H. carbonum*)			
(Race I)						
6	38.9– 99.1	69.5	10.6–14.2	13.5	3 – 12	7.2
7	31.9– 99.1	56.9	10.6–17.7	13.0	3 – 10	6.4
8	24.8– 95.6	57.1	7.1–17.7	12.4	2 – 11	6.8
Means for race I		61.2		13.0		6.8
(Race II)						
9	24.8–102.7	65.9	10.6–14.2	12.7	3 – 12	7.7
10	35.4–102.7	65.2	10.6–17.7	14.3	3 – 11	6.9
11	31.9– 88.5	60.9	10.6–17.7	13.6	3 – 11	7.0
Means for race II		64.0		13.5		7.2
Grand means		62.6		13.3		7.0

those for the northern collections by means of frequency polygons in figure 2, A. In figure 2, B, the spore length distributions of race I and race II of the northern collections are illustrated.

The difference of 26.6 μ between the mean spore lengths of the northern and the southern collections is statistically significant. The difference of 2.8 μ between mean spore lengths of race I and race II is not statistically significant.

Comparisons between the spores of the northern collections with those of the southern collections in respect to such characters as color and curvature emphasize further differentiation. These differences are illustrated in figure 3. Spores from southern collections show considerable curvative as compared with the relatively straight spores from collections of the Corn

Belt species. Spores from southern isolates are fuliginous to olivaceous in color, whereas those from the Corn Belt species are dark olivaceous-brown. All comparisons were made on material of the same age collected from infected leaves of one single cross susceptible to all isolates.

With the exception of *Helminthosporium maydis* Nisik. and Myke. and *H. zeicola* Stout, the species of *Helminthosporium* under consideration bears little resemblance to any of the other species found on corn or other grass hosts. The differences between it and *H. maydis* as pointed out in this paper

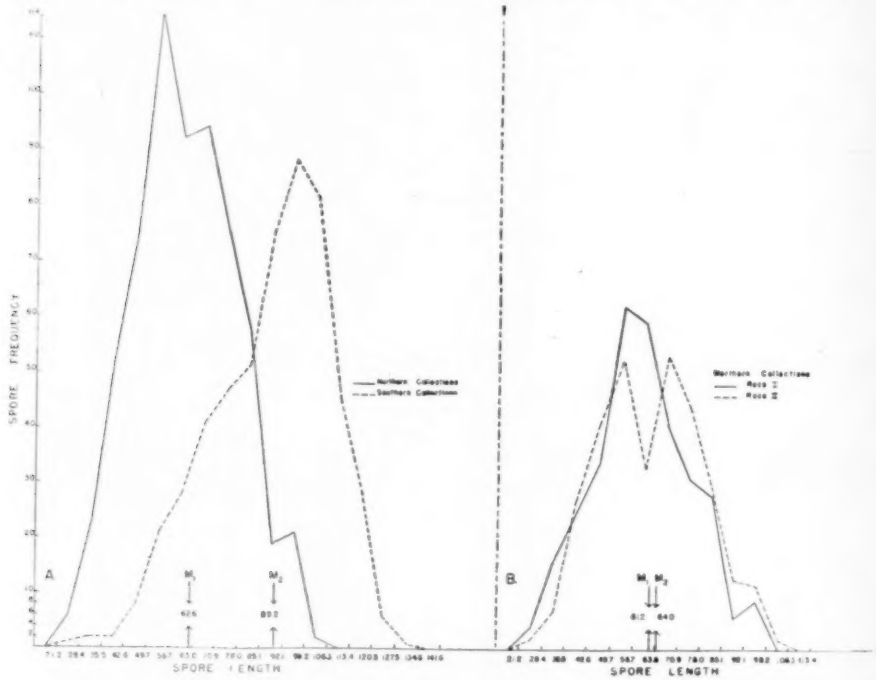

FIG. 2. A. Frequency distribution of spore length of northern collections (*Helminthosporium carbonum*) compared with that of southern collections (*H. maydis*). M_1 (62.6 μ) = mean length of spores from northern collections. M_2 (89.2 μ) = mean length of spores from southern collections. The difference between these means is statistically significant. B. Frequency distribution of spore length of race I and race II of northern collections (*H. carbonum*). M_1 (61.2 μ) = spore length of race I. M_2 (64.0 μ) = mean spore length of race II. Difference between M_1 and M_2 is not significant.

appear sufficient to set it apart as a distinct species. *H. zeicola* Stout, was described in 1930 (5) and similarities between it and *H. maydis* were brought out in the description. Stout was of the opinion that the absence of a perithecial stage and caulicolous habit of the fungus justified specific separation.

Although the virulence of the fungus was not verified, Stout felt that *Helminthosporium zeicola* was pathogenic. Examination of an herbarium specimen of *H. zeicola* was of little value for comparison with the Corn Belt species of *Helminthosporium*. The old cornstalk, while showing some breakdown of tissues at a lower node, did not present any distinct set of symptoms

comparable to that found on host material infected by the species of *Helminthosporium* under consideration. Typical spores of the genus *Helminthosporium* were found associated with the nodal tissues of the specimen, but these were so few in number and in such a collapsed condition that any comparisons could not be considered reliable.

In view of the absence of any descriptions fitting the species of *Helminthosporium* under study and the differences pointed out between it and *H. maydis* and *H. zeicola*, the two species it most closely resembles, a new species, *H.* **carbonum** n. sp. is proposed. The specific name is descriptive

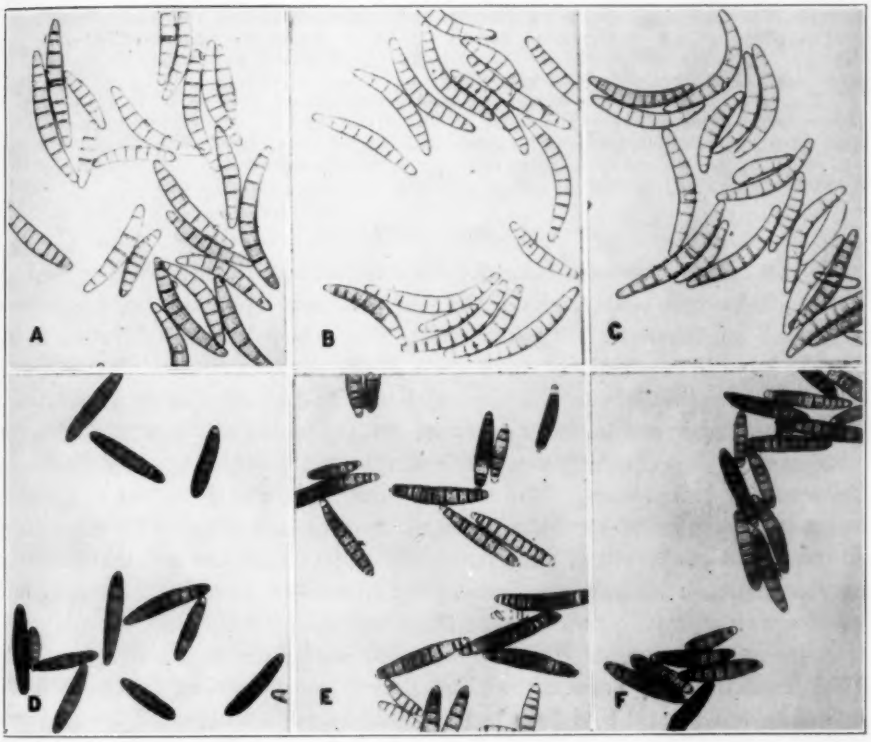

FIG. 3. A–C. Photomicrographs of spores of *Helminthosporium maydis* from three southern isolates. D–F. Photomicrographs of spores of *H. carbonum*. D and E, race I; F, race II. All photomicrographs are of spores collected from infected seedlings of K61 × Pr. Exposures and printing time identical for all illustrations. All magnifications approximately × 230.

of the distinct and characteristic charred appearance of ears of corn infected by the fungus.

Helminthosporium **carbonum** sp. nov.

Conidiis elongato-ellipsoideis, plerumque rectis vel rarissime leniter curvatis, olivaceo-brunneis, 25–100 × 7–18 μ, plus minusve circa 62.6 × 13.2 μ, 2–12 septatis, hilo obscuro; conidiophoris solitariis vel in gregibus e stomatibus erumpentibus, 90–230 × 5–7 μ obscuro olivaceo-brunneis.

Conidia 25–100 × 7–18 μ (means = 62.6 × 13.2 μ) widest in the center and tapering slightly toward rounded ends, straight or slightly curved, dark olivaceous brown, 2–12 septate (mean = 7) with hilum rather inconspicuous, and germinating by two polar germ

tubes. Conidiophores arising singly or in small groups from the stomata, 90–230 × 5–7 μ, dark olivaceous-brown, bearing one to several conidia.

Causing spots on aerial parts of *Zea mays* L. in central United States.

Type specimens deposited in the Arthur Herbarium, Purdue University, Lafayette, Indiana, and in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

The species is divided into two morphologically indistinguishable races, separable on the basis of symptoms produced and specialization in parasitism of one of these races toward certain inbred lines of corn.

Distribution: On aerial parts of *Zea mays* L. race I found throughout central Corn Belt from Iowa to Ohio and from the Ohio River to northern Indiana. Distribution dependent upon occurrence of susceptible inbred lines of corn. Race II collected from Iowa to Ohio and from Tennessee to southern Wisconsin.

Symptoms: Race I: Lesions small, pale-green or yellowish in early infections. Fully developed lesions show zonate pattern, dry light brown centers, light to purplish-brown margins. Lesions range in size from hardly visible spots to those 5 × 20 mm. Fruiting of fungus abundant on leaf sheaths and husks under humid conditions. Ears infected through tips, shanks, or directly through husks. Diseased ears covered with black mycelium imparting a charred appearance.

Race II: Lesions in early stages not unlike those caused by race I. Fully developed lesions are elongated, irregular and up to 3 × 20 mm. in size; chocolate-brown in color, zonate pattern not as distinct as in lesions caused by race I. Race II not highly specialized in parasitism toward inbred lines of corn. Corn ears more frequently attacked by this race than leaves. Symptoms on ears no different than those caused by race I.

DISCUSSION

When *Helminthosporium carbonum* was first studied, it was identified as *H. maydis* because of its similarity to the latter in morphology and symptoms produced on the host. Further studies, in which viable cultures of *H. maydis* have been compared with those of *H. carbonum*, have brought out differences between them which are believed to justify specific separation. The ranges of spore length and spore width, which are common criteria employed in descriptions, are not indicative of morphological differences between the two species. The mean values, particularly of spore length, bring out striking differences between the two fungi. Other differences such as color and curvature of the spores, characters that are not amenable to easy comparison with descriptions in the literature, have been brought out on observation of viable material of these two species.

Original isolations of *Helminthosporium carbonum* race I were made in 1938 from the dent corn inbred line Pr. Since that time two additional inbred lines K61 and K44 have been found to be susceptible. Both of these lines arose from a common stock, the open-pollinated variety Pride of Saline. Single crosses between any two of the three known suscepts are readily infected with isolates obtained from the inbred lines, indicating that the single recessive gene governing susceptibility is identical in all three lines. The dominant allelomorph has been found present in all other lines tested. It appears very probable that with continued development of new inbred lines other susceptible lines will be found. Susceptibility may well be overlooked if the material is grown in an environment unfavorable for the development of the disease. This appears to be the situation with the inbred line Pr. This line originated in Iowa where the disease did not occur, and only recently has the organism been recovered from specimens collected in that State. Obviously, if conditions there had favored the disease, the inbred line

would have been eliminated by either artificial or natural selection. After the line was brought to Indiana, in an environment as optimum for the disease as has occurred since 1938, susceptibility became apparent.

Helminthosporium carbonum race II was first observed in 1939 attacking the leaves of a proprietary inbred line. Since that time no further occurrence of leaf infection has been found in the field. Each year since that time a number of inbred lines have shown ear infections. The inbred line 187–2 appears to be particularly susceptible to ear infection. In general this race is not nearly so virulent nor so highly specialized in parasitism as race I.

During the five years that *Helminthosporium carbonum* has been under observation no indication of an ascigerous stage has been found. Diseased host material held under various environments in the laboratory as well as material subjected to the natural vicissitudes of field conditions have failed to produce a perithecial form that could be associated with the parasite under study. Likewise, a search for hosts of the fungus other than corn, has, to date, yielded negative results.

Original specimens of *Helminthosporium maydis* supplied by Mr. George Y. Young and collected in Southern States during the summer of 1942 were placed in moist chambers in an attempt to initiate the development of the ascigerous stage, *Cochliobolus heterostrophus*. A few perithecia were produced on some of the diseased specimens, but these did not reach full maturity, although held for fifty days in a humid atmosphere. The stage of development reached, however, was sufficient to make identification reasonably certain. The asci containing long ascospores in typical helicoid arrangement were clearly in evidence.

SUMMARY

Further studies are reported on a species of *Helminthosporium* attacking corn. This species, originally identified as *H. maydis*, is shown to be morphologically different from the latter. The differences in morphology brought out on comparison of viable cultures of both species, is based primarily on mean length, curvature, and color of the conidia. Differences between the species in symptoms on seedlings and specialization in parasitism are point out.

The binomial *Helminthosporium carbonum* Ullstrup is proposed; the specific term referring to the charred symptoms characteristic of corn ears infected by this fungus.

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A SEEDLING BLIGHT OF CASTOR BEAN, RICINUS COMMUNIS

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(Accepted for publication September 9, 1943)

Castor beans of the Conner variety planted in crocks of quartz sand in the greenhouses of the Plant Industry Station, Beltsville, Maryland, in the spring of 1942, showed a high percentage of disease at germination. The growing points of many of the diseased seedlings rotted before emergence,

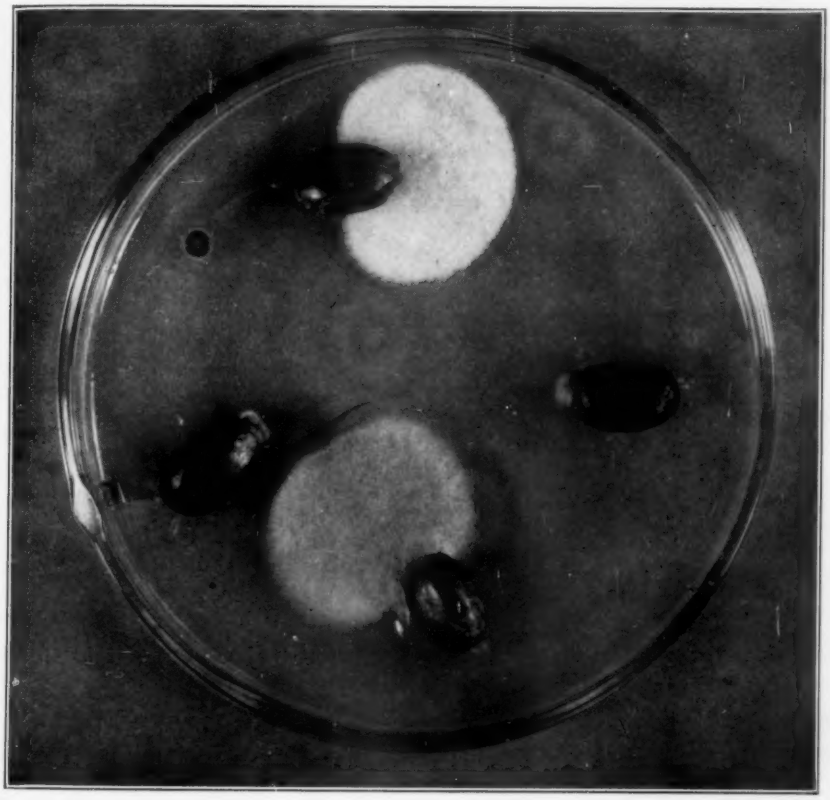

FIG. 1. Growth of *Alternaria* from castor beans of the Conner variety from Urbana, Ill., 5 days after planting surface-sterilized seeds on agar. Natural size.

or the seedlings were killed back soon thereafter. Frequently the hypocotyl rotted off just below the cotyledonary leaves. Other plants, although not killed back, suffered severe injury to the cotyledonary leaves. No spotting of the true leaves was observed, possibly because the humidity in the greenhouse did not favor such infection. Isolations from infected cotyledonary leaves and young shoots consistently yielded an *Alternaria*.

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Since the seeds had been planted in clean sand, it seemed likely that the disease was seed-borne. Accordingly, isolations were made from surface-sterilized seed from the same source (seed of the Conner variety produced by the Department of Agronomy, University of Illinois, Urbana) and from other varieties produced there and at the Plant Industry Station, Beltsville, Maryland, as well as one variety from an unknown source (Table 1). The majority of the cultures were obtained from the caruncle (Fig. 1). In a few instances *Alternaria* also was found when the seed coat was broken open and the endosperm and embryo were removed aseptically. Microscopic examination of seeds revealed the presence of mycelium throughout the caruncles but not elsewhere.

Nine lots of seed were tested for infection by culturing and by planting in sterile white sand in the greenhouse. Seed from 5 of the 9 lots yielded

TABLE 1.—*Isolation of Alternaria from castor bean seed in comparison with the occurrence of seedling necrosis in comparable lots of seeds. This seed was produced in 1941*

Variety	Seed source	Isolation from seeds		Seedling necrosis		
		No. of seeds	No. of seeds yielding *Alternaria*	No. of seeds planted	No. of seed-lings	No. of seed-lings with necrosis
Doughty No. 11	Urbana	25	0	12	12	0
U. S. No. 4	"	15	0	....	....	....
U. S. No. 4	Beltsville	25	0	12	11	0
Conner	"	25	0	12	12	0
Conner	Urbana	95	27	12	10	10
Kentucky No. 38	"	10	4	12	9	3
U. S. No. 7	"	10	3	12	12	0
Kansas Common	"	10	5	12	12	4
Conner Improved	Unknown	20	7	12	10	0

Alternaria on culturing. Seedlings grown from 3 of these 5 lots exhibited necrosis typical of injury by this species. On the other hand, seedlings grown from the 4 lots of seed that did not yield *Alternaria* in culture, did not exhibit Alternaria necrosis (Table 1).

Seeds of the Conner variety produced both at Beltsville and at Urbana were planted in sterile quartz sand, sterile bank sand, sterile gravel, sterile soil, and unsterile soil to determine whether the type of medium in which the seedlings were grown had any pronounced effect on the severity of the disease in the seedlings (Table 2). The disease appeared in a higher proportion of the seedlings grown in quartz sand and in gravel.

In order to determine the effect of seed maturity on seed infection and whether seed infection could occur through inoculated capsules, greenhouse-produced seed of the Conner variety in 3 different stages of maturity, as well as mature Beltsville, field-produced, seed were used for inoculation tests. The stages of maturity were: (a) capsules very green and immature

TABLE 2.—*Comparative performance of Alternaria-infected (Urbana) and Alternaria-free (Beltsville) castor bean seed of the Conner variety when grown in soil, sand, and gravel*

Medium	Seed source	No. seed planted	No. seedlings	No. seedlings with necrosis
Sterile quartz sand	Beltsville	12	11	0
	Urbana	12	12	9
Sterile bank sand	Beltsville	12	11	0
	Urbana	12	12	2
Sterile gravel	Beltsville	12	10	0
	Urbana	12	8	8
Sterile soil	Beltsville	12	12	0
	Urbana	12	11	1
Unsterile soil	Beltsville	12	11	0
	Urbana	12	12	2
Totals	Beltsville	60	55	0
	Urbana	60	55	22

with the spines still pink and the seed and caruncles very succulent and easily injured; (b) capsules nearly mature, just beginning to turn brown, and a few of them beginning to split; (c) capsules mature, brown, and beginning to crack open, with spines very stiff and seed coats and caruncles very hard; (d) mature field-produced, cut September 23, 1942, after a period of heavy rain and dried in a warm greenhouse until December 4, 1942. One-half of each of the lots was shelled and the other half was left in the capsules. The various lots were dipped in a suspension of spores and mycelium of one of the Alternaria isolates and then placed in moist chambers. After 3 days in the moist chambers mycelial growth was abundant on the seeds and capsules, and the lids were removed and the seeds allowed to dry. Five days later the seeds that had been inoculated in the capsules were shelled out, washed in tap water, surface-sterilized, and planted on agar plates. Infection (Table 3) was present in seeds inoculated at all 4 stages of maturity. The appearance

TABLE 3.—*Results from inoculating seeds of the Conner variety of castor bean at various stages of maturity, by dipping the whole capsules or the shelled seeds in a suspension of spores and mycelium of Alternaria*

Where produced	State of maturity	Treatment	Condition when inoculated	No. seeds	No. seeds with *Alternaria*
Greenhouse	(a) Green	Inoculated	Shelled	26	5
			In capsules	28	10
	(b) Partly mature	Not inoculated	Shelled	46	0
			In capsules	52	0
		Inoculated	Shelled	45	5
			In capsules	48	18
	(c) Mature	Inoculated	Shelled	50	10
			In capsules	48	2
Field	(d) Mature	Not inoculated	Shelled	27	1
			In capsules	41	1
		Inoculated	Shelled	27	0
			In capsules	48	5

of some *Alternaria* in the noninoculated field-produced seed suggests slight earlier infection of these seeds. Nearly all of the growth of *Alternaria* from the seeds was from the caruncles. Occasionally there was some growth from other parts of the seeds, particularly from injured seeds, which were common in the very young, succulent material. Mycelium was present throughout the caruncles of all of the young, succulent seeds and was abundant in the nucellus. The endosperm of these young seeds had shriveled so much that the seeds were very light weight. Whether this was due to infection by the *Alternaria* or to the removal of the spikes from the plants before maturity was not determined. In the more mature seeds the mycelium was not extensive in the caruncles, being confined largely to the outer cells. A

FIG. 2. Castor bean seedlings of the Conner variety inoculated with a spore and mycelial suspension of *Alternaria compacta* at the time of emergence (right). Uninoculated seedlings (left).

Fusarium was present in a large percentage of the caruncles of the field-produced seed.

The pathogenicity of the *Alternaria* isolated from castor-bean seeds was further established by pouring a suspension of spores and mycelium over seedlings just beginning to break through the surface of sterile quartz sand. Four of 12 seedlings so inoculated were dead 14 days after inoculation; 7 others exhibited many mild to severe necrotic lesions (Fig. 2). Infection also occurred when plants 3 weeks old were sprayed with a spore and mycelial suspension and placed in a moist chamber for 4 days. Large areas of the inoculated leaves became water-soaked and flaccid (Fig. 3) and all were covered with numerous slightly zonate, dark-brown necrotic lesions approximately one-fourth inch in diameter (Fig. 4). Many of the leaves

were shed, but no stem lesions were observed. Control plants sprayed with water only and placed in the moist chamber at the same time remained healthy. Similar but less numerous and smaller lesions were observed on more mature plants inoculated at the same time.

Macrosporium leaf spots on *Ricinus communis* L. have been reported from Korea and Japan (10), from southern Russia (6, 8), and from Italy (1, 7). Tropova (8) observed losses up to 20 per cent in *R. communis* plantings due to *Macrosporium cavarae* in the Don region of Russia in 1926. Reports of leaf spots caused by *Alternaria* in Texas, Florida, and Louisiana have appeared in the Plant Disease Reporter (9), and *Alternaria brassicae*

FIG. 3. Foliage of 21-day-old castor bean plants 4 days after inoculation with a spore and mycelial suspension of *Alternaria*. Healthy plants on the right.

is listed as causing a leaf spot on old plants of *Ricinus* in New York. *Alternaria* species also have been reported as causing leaf spots on *Ricinus* in Brazil (2) and in India (3). Baldacci (1) isolated *M. cavarae* and an undetermined *Fusarium* from the apices of *R. communis* seeds. He believed that the former causes a leaf spotting and attacks the apices of seeds (presumably the caruncles), whereas the *Fusarium* was thought to produce lesions on the stems, inflorescences, capsules, and seed apices.

Three species of *Macrosporium* have been described on *Ricinus communis*. Yoshii (10) described a *Macrosporium* pathogenic on castor bean leaves, which he named *M. ricini*. The spores are 42 to 78 μ long by 8.7 to 19.5 μ wide with long (30–100 μ), slender beaks, whereas the spores of the writer's organism have no elongate beaks and are smaller (14.0–38.5 μ × 7.0–19.5 μ with a mean of 21.4 μ × 10.8 μ). Parisi (7) described *M. cavarae*, which

produces round, brownish, slightly zonate leaf spots. She reports that the cotyledons and first leaves of seedlings were attacked with considerable virulence but she did not report pre- or post-emergence killing of the seedlings such as the writer has observed. The spores are described as being clavate, 34–40 μ × 10–13 μ, and having a "pedicel." Ravenel collected a fungus that was fruiting on mature stems of castor beans at Houston, Texas, in 1869, and was later described by Cooke (4) as *M. compactum*. He states

FIG. 4. Young castor bean leaf 4 days after inoculation with *Alternaria compacta*. × ⅓.

that the spores are obtuse and measure 20–30 μ × 12–14 μ. However, measurements of 12 spores from herbarium type material show a range of 15.8–38.5 μ × 7.0–17.5 μ. This is in good agreement with measurements of spores from the writer's isolates. Cooke made no mention of a leaf spot in his brief description. On the other hand, the writer has not observed *Alternaria* fruiting on the stems of his material; but, because of the similarity in the measurements and the appearance of the spores, the writer's organism is considered to be the same as the one described by Cooke as *M. compactum*. Because of the spore size and shape, *M. compactum* falls

into the *Alternaria tenuis* group of Elliott (5) and becomes **A. compacta** (Cooke) n. comb. Herbarium material of *M. cavarae* was not available for study, but it is thought to be also synonymous with *A. compacta*, since Parisi's description of the symptoms on the foliage and seedlings is very similar to the writer's observations on the disease described herein. The spore measurements she presents show *M. cavarae* as having slightly longer spores (34–40 μ), but this is not thought to be sufficient to warrant separation from *A. compacta*.

SUMMARY

A seed-borne disease of castor beans caused by a species of *Alternaria* is described. It causes pre- and post-emergence damping-off and a seedling and foliage blight, and readily infects immature seeds.

Three species of *Macrosporium* previously described on *Ricinus communis* are discussed and their descriptions compared with the writer's isolates. The writer's isolate is believed to be distinct from *Macrosporium ricini* Yoshii but identical with *M. compactum* Cooke and *M. cavarae* Parisi, which are here given the new designation **Alternaria compacta** (Cooke) n. comb.

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RELATION OF SPORE DIMENSIONS TO THEIR RATE OF FALL

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The rate at which fungus spores fall through still air constitutes an important primary element in the understanding of the possibilities of their dissemination by the wind, both locally and over long distances. Long-distance travel of spores must be taken into special account in plant-quarantine activities, because successful intercontinental transport of spores via air drifts and prevailing winds might in some cases nullify attempts to exclude foreign diseases by the customary port-inspection methods. In the study here presented an attempt was made to establish from available information on the rate of spore fall in still air a relation to spore dimensions that will permit the probable rate of fall of any spore to be determined with a fair approximation to accuracy.

Any constant relation between spore dimensions and rate of fall is likely to be reached through the spore surface, since air friction, and consequently retardation, is largely dependent on the superficial area of the falling spore. Spore surface is obtained from the formula πd^2, which becomes $\pi(l \times w)$ for oval or cylindrical spore types by substitution of the geometric mean diameter, $\sqrt{l \times w}$, for d. Since the value π is a constant, any correlation between rate of fall and spore surface must be sought through the variable $(l \times w)$ or its equivalent d^2.

The actual rates of fall of the spores of some 20 fungi have been determined experimentally,[2, 3, 4] and as the spore dimensions of these are also available the desired correlation can be attempted. Table 1 gives the determined rates of fall for these 20 spore types together with spore dimensions and other derived values needed for correlation. If the $(l \times w)$ values (microns) for these spores, and their rates of fall (mm. per sec.) are plotted independently on the same graph, each against spore diameters (microns), there are obtained two curve series of apparently similar type but not coincident. To bring the $(l \times w)$ values into a common position on the graph with the rate-of-fall series it is necessary to introduce a suitable constant. This constant is readily obtained by finding the average of the values $\frac{l \times w}{\text{rate of fall}}$ as determined for each spore. For reasons that will appear later the spore last listed in table 1, *Helminthosporium sativum,* was omitted from the calculation; the average for the remaining 19 (Table 1) was found to be very close to 40 (39.67).

In the graph in figure 1 the rate of fall for each of these 19 spores, expressed in millimeters per second, has been plotted against spore diameters

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[2] Christensen, J. J., in Aerobiology, A.A.A.S., Pub. No. 17, 1942; p. 81.

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[4] McCubbin, W. A., Phytopath. 8: 35–36, 1918.

in microns. There has also been included a curve representing an arbitrary series of the values $\frac{l \times w}{40}$ or their equivalents, $\frac{d^2}{40}$, likewise plotted against spore diameters, the perpendicular scale thus serving to represent millimeters per second for the rate-of-fall series and microns for the derived curve. It will be observed that there is a reasonably good coincidence between this curve and the points representing the determined rate-of-fall values. Insofar, therefore, as this coincidence may be relied on to represent

TABLE 1.—*Spore dimensions, observed rates of fall, and values derived from these, used in preparing the graph (fig. 1)*

Fungus	Spore dimensions (microns) (l)	(w)	Product of spore dimensions ($l \times w$)	Square root of product $\sqrt{l \times w}$	Observed rate of fall (mm. per sec.) r	Ratio $(l \times w) \div r$
Collybia dryophila	5.44	3.23	17.57	4.192	0.49	35.86
Pluteus cervinus	5.95	4.57	27.19	5.214	0.67	40.58
Paxillus involutus	7.48	4.88	36.50	6.042	1.10	33.18
Psalliota campestris (field)	7.26	5.35	38.84	6.232	1.06	36.64
Psalliota campestris (bed)	9.7	5.80	56.26	7.500	1.61	34.94
Marasmius oreades	9.5	5.6	53.20	7.300	1.34	39.70
Boletus badius	12.8	4.29	54.91	7.410	1.09	50.38
Amanita rubescens	9.38	6.53	61.25	7.826	1.54	39.77
Galera tenera	10.47	6.06	63.44	7.965	2.13	29.79
Russula emetica	8.82	7.5	66.15	8.133	1.64	40.33
Polyporus squamosus	14.6	5.13	74.89	8.654	1.03	72.72
Ustilago zeae	10.0	9.0	90.00	9.487	3.50	25.71
Coprinus comatus	12.55	7.48	93.87	9.688	3.96	23.70
Amanitopsis vaginata	10.87	10.87	118.15	10.870	2.95	40.05
Coprinus plicatilis	12.9	7.9	101.91	10.092	4.29	23.75
Alternaria sp.	20.0	10.0	200.00	14.142	3.00	66.67
Cronartium ribicola	22.0	19.0	418.00	20.445	10.00	41.80
Puccinia graminis tritici	28.0	17.0	476.00	21.817	12.00	39.67
Puccinia triticina	25.0	20.0	500.00	22.361	13.00	38.46
Helminthosporium sativum	75.0	20.0	1500.00	38.730	20.00	75.00

reality, a relation is established between simple spore dimensions and observed rates of fall, which may be used to predict the probable rate of fall of any spore in this type group, which includes spherical and oval spores as well as those of cylindrical shape with hemispherical ends. The formula expressing this relation is: $\frac{l \times w}{40} = r$, where l is spore length in microns, w the breadth in microns, and r the rate of fall in millimeters per second.

Spores of fusiform type cannot be incorporated directly into this simple formula series. In computing the surfaces of the two cones set base to base, which make up the simplest type of fusiform spore, one has to employ the slant side of the cone, a value that does not vary directly with the spore dimensions l and w. Moreover, some types of fusiform spores are not con-

structed on the double-cone pattern, but may be built on the model of a cylinder set between two cones, the length of the intercalated cylinder usually having no fixed relation to the total spore length, l.

The surfaces of both types of fusiform spores can be readily calculated, however, and if it is assumed that falling rate here has the same relation to spore surface as in the case of spherical, oval, or cylindrical spores, then one can easily obtain a formula to cover each case.

For double-cone spores this formula is $\frac{w \times s}{40} = r$, where w is spore width, s the slant side of one cone element, and r the rate of fall in millimeters per

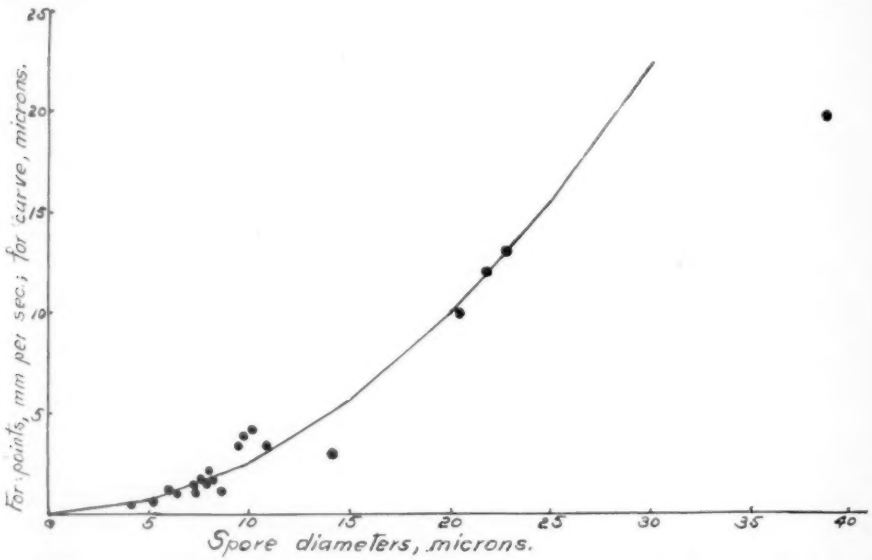

FIG. 1. Observed rates of fall (mm. per sec.) for the spores listed in table 1 are plotted against spore diameters (microns) to give the point series. For the curve, spore diameters, d, of a hypothetical series of spherical spores are plotted against $\frac{d^2}{40}$ (microns) to indicate that the value of $\frac{d^2}{40}$ in microns is closely comparable with the rate of fall in millimeters per second.

second. In terms of the ordinary spore dimensions, l and w, this becomes $\frac{w \times \sqrt{l^2 + w^2}}{80} = r$.

For most ordinary needs where the mathematical accuracy of this formula is not required, the simple formula $\frac{l \times w}{80} = r$ will give an approximately correct falling rate. It merely substitutes the axial cone length $\frac{l}{2}$ for the slant side s, on the assumption that in slender spores these values are so nearly identical that the error introduced by the substitution will be very small.

A more accurate empirical formula substitutes for s the axial cone length plus a variable fraction expressed in terms of l and w, which thus gives a value approximately equal to s. This formula is:

$$\frac{w\left(l+\frac{w^2}{2l}\right)}{80}=r$$

This formula is surprisingly accurate for spores in which the ratio of l to w is above 2:1.

For fusiform spores consisting of two cones and an intercalated cylinder of the same axial length as each cone element, that is, cylinder length $=\frac{l}{3}$, the mathematical formula for falling rate is:

$$\frac{w\left(\sqrt{\left(\frac{l}{3}\right)^2+\left(\frac{w}{2}\right)^2}+\frac{l}{3}\right)}{40}=r$$

For the same spore type the empirical formula is:

$$\frac{w\left(\frac{2l}{3}+\frac{3w^2}{8l}\right)}{40}=r$$

And the simple approximate formula is: $\frac{l\times w}{60}=r$

For the general fusiform type of spore it is necessary to introduce another symbol, x = axial length of one cone, to obtain this formula:

$$\frac{w\left(\sqrt{x^2+\left(\frac{w}{2}\right)^2}+l-2x\right)}{40}=r$$

The corresponding empirical formula is:

$$\frac{w\left(l-x+\frac{w^2}{8x}\right)}{40}=r$$

and the simple approximate formula $\frac{w(l-x)}{40}=r$

It will now be apparent that the spores of *Helminthosporium sativum* were omitted from the initial calculation because they fall into another spore group from the present point of view, belonging somewhere in the fusiform types, which have somewhat different relations of surface to the dimensions l and w. If one considers that these spores come fairly close to the type represented by two cones and a central cylinder of about cone length, the calculated falling time would be 26 mm. per second, according to both the mathematical and empirical formulae and 25 mm. per second for the approximate formula. The observed falling time was 20 mm. per second.

In presenting this study of the relation between ordinary spore dimensions and their rate of fall, it is emphasized that no high degree of accuracy should be expected from the formulae given; they are derived from the average values of a scanty series of determinations; hence, the probability

of error is high. It is hoped that when additional spore-fall determinations are available a recheck of these relations will bring something closer to reality. Also these formulae leave out of consideration the changing relation of spore surface to spore volume among the members of a size series of spores. With size increase goes an increased volume per unit of spore surface, resulting in greater gravitational pull earthward in proportion to air resistance. Nor is it wise to overvalue the rate of spore fall that can thus be predicted. Other factors outside the scope of this prediction method, such as spore density, peculiarities of shape, and irregularities in surface configuration, introduce such great variations into the rate of fall that prediction may become in some cases uncertain or of diminished worth. One may be confident, however, that with all these shortcomings the rate of spore fall will always serve as a sound and orderly point of departure for estimating the possibilities of long-distance spread of pathogens in air currents. When these extremely slow rates of fall are contrasted with the relatively imposing velocities attained by wind movements or even by slight convection currents, it is apparent that the feeble tendency of fungus spores to drift earthward under the pull of gravity can have very little effect in preventing their dissemination in favorable air currents for very long distances over the earth's surface.

In closing it may be mentioned that this spore-fall problem should constitute an excellent student exercise, serving in simple fashion to present a concrete picture of spore movement in still air, and particularly to direct attention to the altered physical relations that obtain in the size plane of fungus spores.

A TECHNIQUE FOR TESTING RESISTANCE OF COTTON SEEDLINGS TO THE ANGULAR LEAF SPOT BACTERIUM

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In the course of pathogenicity tests with isolates of *Phytomonas malvacearum* (E.F.S.) Bergey, the cause of angular leaf spot of cotton, indications were obtained that seedlings might behave in a manner similar to mature plants with respect to varietal resistance against the organism. The technique presented here was developed to test this hypothesis. In preliminary experiments inoculation of seed prior to planting proved far superior to seedling inoculation in furnishing varietal differentiation with respect to disease resistance. Similar results have been reported for bacterial blights of beans.[2,3]

It would obviously be desirable to choose for such a study a series of varieties of known resistance. Unfortunately no critical field data were available, except the well-established evidence that varieties of Egyptian cotton, *Gossypium barbadense* L., are much more susceptible than varieties of upland cotton, *G. hirsutum* L. The materials used here were selected on the basis of two field trials conducted at Clemson, South Carolina, in 1942. Plants of 20 varieties and lines were inoculated by spraying with bacterial suspensions according to the method of Knight.[4] The following 5 varieties[5] are representatives of the range of resistance exhibited by the 20 varieties in the field: S × P Egyptian (extremely susceptible), Shafter Acala (highly susceptible), Rogers' Acala (moderately susceptible), Stoneville 4–5 (tolerant), and Stoneville 4–8 (resistant). Seed lots of these 5 varieties were used with the method to be described.

PRINCIPAL FEATURES OF THE METHOD

1. Reaction is tested separately to "external" and "internal" inoculation of seed, by immersing them in bacterial suspensions for short and for long periods.

2. Seedlings are grown for three weeks under conditions favorable for infection of cotyledons, i.e., abundant water supply and high temperature.

[1] Agent (Associate Pathologist), Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, in cooperation with the South Carolina Agricultural Experiment Station.

[2] Zaumeyer, W. J. The bacterial blight of beans caused by *Bacterium phaseoli*. U. S. D. A. Tech. Bull. 186. 1930.

[3] Stapp, C. Verfahren zur Prüfung von Bohnen (*Phaseolus vulgaris*) auf Resistenz gegen *Pseudomonas medicaginis* var. *phaseolicola* Burkh., den Erreger der Fettfleckenkrankheit. Angew. Bot. 15: 241–252. 1933.

[4] Knight, R. L., and T. W. Clouston. The genetics of black-arm resistance. Jour. Genetics 38: 133–159. 1938.

[5] In order to simplify the discussion, varieties and lines are designated here as varieties. For kindly supplying the seed lots thanks are due to C. J. King, G. J. Harrison, C. H. Rogers, and D. M. Simpson. Stoneville 4–5 and 4–8 are lines obtained from Mr. Simpson. The delinted seeds of the lots were free of seed-borne pathogens.

3. Relative disease rating is based on severity of lesions as well as on rapidity of their development.

Procedure

A suspension of a recent isolation of *Phytomonas malvacearum* was prepared from a streak culture grown for 4 days on potato-dextrose agar in a Petri dish. The bacterial masses were suspended in 600 cc. of distilled water and filtered through 4 layers of cheesecloth. By means of dilution cultures the number of viable bacteria was estimated at 2.5 million per cc.

Inoculation of seed was carried out by immersing them in the bacterial suspension, using 25 cc. for each sample of 60 acid-delinted seed. One

FIG. 1. Cotyledons of cotton seedlings infected with *Phytomonas malvacearum*. Degrees of severity from left to right: control, slight, severe, very severe, practically destroyed. Last row at right is S × P Egyptian; the others are Shafter Acala. Photographed 6 days after the seeds were inoculated and planted. Natural size.

sample of each variety was left in the suspension for 5 minutes, the other for 3 hours at 24–26° C. The flasks were agitated at intervals. Finally, the suspensions were decanted and the seeds were dried on paper toweling.

The seeds were placed between layers of paper toweling that were kept moist by standing in tumblers partly filled with water. This method is a modification of techniques described by Simpson, *et al.*[6] A towel consisted of two thicknesses of paper and measured 13.5 × 34.0 cm. Twelve seeds were arranged on the moistened towel 2 cm. from one margin, leaving one-half of the towel free to be folded over them. The resulting rectangle of 13.5

[6] Simpson, D. M., *et al.* Anatomical structure of the cottonseed coat as related to problems of germination. U. S. D. A. Tech. Bull. 734. 1940.

×17.0 cm. was placed against the inner wall of a glass tumbler of 300 cc. volume. The seeds were held by the toweling at the upper edge of the tumbler with the 2 cm. margin extending beyond the glass. The paper towel thus formed a wick during seed germination and allowed the roots to grow between the layers toward the bottom of the tumbler. A 10×15 cm. filing card was placed within the tumbler in order to hold the toweling appressed to the wall.

No attempt was made to provide aseptic conditions. The towels were thoroughly moistened in distilled water, the excess water being removed by pressure. At the beginning, 50 cc. of distilled water was poured into each tumbler; later, tap water was added when needed to keep the water level at ½–⅔ the height of the tumbler.

The tumblers were kept for 4 days in incubator at 27–30° C. in a saturated atmosphere. On the fourth day the seed coats were removed to prevent development of mold on the towels; when necessary the seedlings were arranged so that the cotyledons were just above the upper margin of

TABLE 1.—*Factors for calculation of disease index of table 2*

Group No.	Symptoms of cotyledons		Factor
	Severity	Day after planting	
1	Practically destroyed	6th	1.0
2	Very severe	"	0.9
3	Severe	"	0.8
4	Slight	"	0.6
5	Severe	11th	0.6
6	Slight	"	0.4
7	"	16th	0.2
8	"	21st	0.1

the towel; and the seedlings were thinned to 10 per tumbler, leaving 50 seedlings of each sample in 5 tumblers. The tumblers were then moved to trays on a greenhouse bench. Air temperatures were held above 27° C. by a heating cable with thermostatic control and below 35° C. by cheesecloth shades, which protected the tender seedlings from the mid-day sun.

On the sixth day after planting, and subsequently at 5-day intervals, seedlings with lesions were removed from the tumblers and graded according to severity of symptoms (Fig. 1 and Table 1). For the purpose of these experiments, the term lesion is used only for typical bacterial spots, dark-green in reflected light and translucent by transmitted light. Computation of the disease index for each sample of 50 seedlings is explained in tables 1 and 2. The frequent removal of lesioned seedlings helped in suppressing secondary inoculation of seedlings from adjoining diseased cotyledons. In order further to preclude such spread, precautions were taken to prevent wetting of cotyledons.

RESULTS AND DISCUSSION

It is evident from table 2 that the seedling data on relative varietal resistance to *Phytomonas malvacearum* agreed well with the field experiments.

Results of a second experiment checked those of the first one in all essential points. Further tests with other varieties and lines have given similar results. The data can thus be safely interpreted as meaning that, in general, varietal reaction of seedlings inoculated by the described method conforms with observations on varietal behavior of field plants. It would seem worthwhile, therefore, to consider the technique from the standpoint of its possibilities as an aid in breeding cotton varieties for resistance to angular leaf spot.

The advantages of the method are obvious: Saving in time and labor, ease and uniformity of inoculation, and possibility of controlling environmental factors. Details of the technique may be considerably simplified or adapted to the material under test. Planting the inoculated seed in pots

TABLE 2.—*Varietal reaction of cotton seedlings to angular leaf spot. Seed inoculation. Seedlings grown for 21 days at 27–35° C. Varieties arranged from left to right in order of increasing disease resistance in the field*

	Period of inoculation	Varieties and lines				
		S × P Egyptian	Shafter Acala	Rogers' Acala	Stoneville 4–5	Stoneville 4–8
Percentage diseased	5 minutes	100.0	92.0	48.0	64.0	18.0
	3 hours	100.0	100.0	86.0	66.0	28.0
Disease index[a]	5 minutes	71.2	42.2	10.2	21.0	5.0
	3 hours	91.0	56.4	47.0	22.2	6.0

[a] Calculation of disease index from a sample of 50 seedlings: The number of seedlings falling in each group of table 1 is multiplied by the corresponding factor. The sum of these products is doubled in order to make 100 the highest disease index possible for the 50 seedlings.

or tumblers with steamed sand was in use even before the more tedious paper-towel method. The latter was adopted because the individual seedlings could be kept under close observation. This is important in the early stages of development when seedlings of very susceptible varieties are severely injured. Comparison of such varieties as S × P Egyptian and Shafter Acala is facilitated by a disease index, such as that of table 2. The more resistant varieties may be fairly well differentiated according to percentage of seedlings affected by disease.

A method of inoculating fuzzy seed by spraying with bacterial suspension[7] was tried and gave results similar to those reported here. For the purpose of these tests acid-delinted seed has the advantage of being practically free of seed-borne pathogens.

The use of 2 periods of inoculation (5 minutes and 3 hours) has distinct value for differentiating upland cottons with respect to varietal resistance (Table 2). When delinted seed is kept in the bacterial suspension for 3 hours, the seeds swell sufficiently to allow the entrance of bacteria, corresponding to seed internally infected in the field before cotton is picked. Submerging the seed in the bacterial suspension for 5 minutes deposits bac-

[7] Unpublished method, obtained from C. J. King.

teria on the seed coat in a manner simulating the natural condition of seed carrying inoculum externally. Attempts to replace the two procedures by a single one have not been promising. An intermediate inoculation period of 30 minutes did not always produce intermediate infection. The large variations in infection obtained from this treatment are probably due to variability in swelling of seed during the period of inoculation.

The reaction of the two Stoneville lines differed from that of the other varieties. The latter became more severely infected following the 3-hour inoculations than after the 5-minute exposure, while the Stoneville lines showed little or no differences. This behavior seemed so puzzling that, in repeating the experiment, the number of replications was doubled for Rogers' Acala and Stoneville 4–5. The results were consistent with those of table 2. A possible explanation was suggested by the frequent appearance of necrotic spots on Stoneville 4–8, when seed was inoculated for 3 hours. Moreover, when seed of this line was kept in the inoculum for 24 hours before planting, typical bacterial lesions were neither more severe nor more numerous than following the shorter exposures, but more necrotic spots developed. Perhaps, the Stoneville 4 lines possess a genetic factor of resistance that involves hypersensitivity of the cotyledons, causing the affected cells to die so rapidly that the parasite has little chance to produce the typical lesions.

SUMMARY

A technique is described for testing resistance of cotton seedlings to the angular-leaf-spot bacterium. Seed was inoculated by immersing in suspensions of bacteria for short and lengthy intervals. Seedlings were grown for 3 weeks at 27–35° C. In general, varietal reaction to the disease organism in these seedling tests conformed with that of field plants. The method offers possibilities as a rapid supplementary test in breeding varieties for resistance to the disease. For this purpose the technique may be adapted to the material under test by modifications, such as the use of sand culture in place of the paper-towel technique described here.

A METHOD OF INDUCING BARK-SHELLING FOR TREATMENT OF CERTAIN TREE DISEASES

H. S. Fawcett[1] and L. C. Cochran[2]

(Accepted for publication August 25, 1943)

There are certain diseases of trees which affect primarily the outer layers of bark and which may be treated in their early stages by eliminating these diseased parts. It is desirable to develop an easily applied method of accomplishing this tissue elimination without too severe injury to the tree and without excessive cost in material and labor.

For the past 20 years or more the senior writer has discussed with his associates and others the desirability of finding a substance that could be safely applied to the bark of citrus trees and would cause the outer half, or more, of the bark to shell off, thus to imitate the standard method of scraping the bark for treatment of citrus trees affected with psorosis (California scaly bark).[3] The difficulty that arose was to find a substance that could be applied to bark of varying thicknesses and accomplish such shelling without causing severe injury where the bark was thin. It is believed that, with certain limitations and reservations, a method has been found for this treatment of citrus trees. The ideal substance sought is one that can be easily applied and will penetrate and kill rapidly only the outer bark tissue but one which would not penetrate and kill the cambium. This would allow the cambium to continue growth and generate new bark promptly. The old bark with its accompanying psorosis lesion would then dry and shell off. The advantage of this method over the standard scraping method would be the greatly reduced cost of labor and increased efficiency and ease of treating crotches and irregular surfaces.

Some experiments involving this method along with others were made as early as 1922,[4] but the first trial of the more recent series of experiments with the present method was made at the Rancho Sespe, near Fillmore, California, in 1937. Dinitro-o-cyclohexylphenol at a concentration of 2 per cent by weight dissolved in dormant-spray oil was applied with a brush to psorosis bark lesions of two Valencia orange trees. After a few months slabs of bark shelled off, exposing newly-formed bark beneath.

In May and June, 1939, a more extensive experiment[5] was carried out at the Los Alisos Ranch near El Toro, California, to compare this and other methods with the standard scraping treatment of psorosis bark lesions on

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[3] Fawcett, H. S. Citrus diseases and their control. 656 pp. 187 figs. McGraw-Hill Book Company, New York, N. Y.

[4] In cooperation with M. B. Rounds, liquor cresolis compositus was used at Azusa, California, and caused some bark shelling.

[5] This was started in cooperation with Paul Sloop and E. T. McFadden. Assistance was given by J. M. Wallace in this and subsequent experiments.

Valencia trees. Dinitro-o-cyclohexylphenol (subsequently to be referred to as DNOCHP) at 2% dissolved in dormant-spray oil was brushed on the lesions of 10 trees, and 40 trees were given the standard scraping treatment.

TABLE 1.—*Degree of injury and of bark shelling from application of various solutions to 15-year-old Washington Navel and Valencia orange trees, 18 months after treatment*

Material	Var.	Trunk		Limbs 2 to 4″ dia.		Limbs Less than 2″	
		Injury	Shelling	Injury	Shelling	Injury	Shelling
Dormant spray oil alone	Nav.	0[b]	0[c]	0[b]	0[c]	0[b]	0[c]
	Val.	0	0	0	0	0	0
⅛% DNOCHP[a] in dormant-spray oil	Nav.	0	0	0	0	0	0
	Val.	0	0	0	0	0	0
¼% DNOCHP in dormant-spray oil	Nav.	0	1	0	0	0	0
	Val.	0	2	0	2	M	1
½% DNOCHP in dormant-spray oil	Nav.	0	1	0	1	0	0
	Val.	0	2	0	2	Sl.	2
1% DNOCHP in dormant-spray oil	Nav.	0	3	0	2	0	1
	Val.	0	3	0	3	Sev.	1
2% DNOCHP in dormant-spray oil	Nav.	0	3	0	3	M	3
	Val.	0	3	0	3	Sev.	1
1% DNOCHP + 2% glycerol mono-oleate in medium grade citrus-spray oil	Nav.	0	3	0	2	0	1
	Val.	0	3	0	3	M	2
2% DNOCHP + 2% glycerol mono-oleate in medium grade citrus-spray oil	Nav.	0	3	0	3	Sl.	3
	Val.	0	3	Sl.	3	Sev.	1
2% DNOCHP + ½% aluminum stearate + 1% glycerol mono-oleate in medium grade citrus-spray oil	Nav.	0	3	Sl.	3	Sev.	2
	Val.	Sl.	3	Sl.	3	Sev.	1
½% Aluminum stearate in medium grade citrus spray oil	Nav.	0	3	0	3	Sl.	3
	Val.	0	3	Sl.	3	Sev.	0
½% DNOCHP in medicinal grade white mineral oil	Nav.	0	1	0	1	0	1
	Val.	0	2	0	1	0	1
1% DNOCHP in medicinal grade white mineral oil	Nav.	0	3	0	3	0	3
	Val.	0	3	0	3	0	2
2% DNOCHP in medicinal grade white mineral oil	Nav.	0	3	0	3	0	2
	Val.	0	3	0	3	0	3
2% DNOCHP in kerosene	Nav.	0	3	0	3	0	3
	Val.	Sl.	3	Sl.	3	0	1
0.8% DNOCHP + 2½% diamyl-phenol in kerosene	Nav.	0	3	0	3	0	2
	Val.	0	3	0	3	0	2

[a] DNOCHP = dinitro-o-cyclohexylphenol.
[b] 0 = no injury; sl., slight inj.; M, med. inj.; sev., severe inj.
[c] 0 = no bark shelling; 1, slight bark shelling; 2, med. but incomplete shelling; 3, complete or nearly complete shelling.

Although some patches of cambium were killed in the chemically treated areas, especially on the limbs where the material was applied heavily, the results were essentially the same as were obtained earlier at the Rancho Sespe.

The bark was painted not only over the lesions, but in a border of about 6 in. above and below and about 3 in. on the sides of each lesion. After a few months slabs of bark including the lesions shelled off, and the cambium, with the exception of a few areas, had produced new bark under the treated areas. Although the 2% DNOCHP in dormant-spray oil was found on some trees to cause too much injury for safe general use, the effects compared favorably, especially on the trunks, with those of the standard scraping method. Whenever the material was allowed to run down to the soil line and collect there, much injury resulted from death of the cambium.

These favorable results, when precautions were taken in application, suggested the possibility of producing the desired bark-shelling without injury to the cambium by decreasing the concentration of the DNOCHP in the oil, or by using oils of other viscosities as carrier solvents, or by using other solute materials. An experiment was set up using 19 different solutions.[6] These were applied in November, 1941, each to one healthy Valencia and one healthy Washington Navel orange tree, 15 years of age, at the California Citrus Experiment Station. The materials were applied to a strip of bark extending from the ground line up the trunk and on to limbs varying in size from about 4 inches down to ½ inch in diameter. In April, 1942, the applications were repeated on this same set of trees on portions of bark not treated the previous November. The results of fifteen of these tests for November, 1941, are shown in table 1, 18 months after treatment. The manner of bark shelling in two of the above tests is shown in figure 1. The results of the April test were similar. Four of the materials that were used are not listed in table 1, since they caused no shelling of bark. These were 2% DNOCHP in cottonseed oil, 1% DNOCHP in boiled linseed oil, 1% DNOCHP in glycerine, and 1% DNOCHP in petroleum jelly. The first three caused neither injury nor shelling, the last caused a very slow injury without drying or shelling, even after 16 months. Table 1 indicates that under the conditions of these tests, DNOCHP in concentrations below about 1% in dormant-spray oil is not effective in inducing good shelling of the bark. In concentrations higher than 1% it tends to cause some injury.

Nine of the most promising of the original 19 materials, as well as kerosene alone, were tried at the Azusa Foothill Ranch in May, 1942, on psorosis lesions of Washington Navel and Valencia orange trees. Five of these materials were selected for a test in November, 1942.[7]

Another test of the same 19 materials and of kerosene alone was made on psorosis lesions on Valencia orange trees at Rancho Sespe in July, 1942. With one exception, results were similar to those on healthy bark at the Citrus Experiment Station, but more injury was caused by the stronger solutions; kerosene alone caused no appreciable effect if not allowed to run down into the soil. Ten of the most promising of these materials were tried in another experiment at Rancho Sespe in October, 1942. Five of these and

[6] Acknowledgment is due Dr. A. M. Boyce, who from his experience with solvents for this compound, helped to devise the formulas and had them prepared for the experiment.

[7] The senior writer was assisted by M. B. Rounds in this experiment.

five additional ones, with lower specific gravity oils as solvents, were tried in December, 1942.[8] The results of the applications of the most promising solutions, such as 1% DNOCHP in kerosene, in gasoline, or in white mineral

FIG. 1. A. Normal bark of navel orange treated with 2% DNOCHP in kerosene. B. Normal bark of Valencia orange treated with 2% DNOCHP in white mineral oil. New bark can be seen where the old bark scales have fallen away.

[8] The Rancho Sespe, through Howard Pressey and T. A. Lombard, gave much assistance in carrying out these experiments.

oil, to bark containing psorosis lesions showed that the cambium under the lesion is somewhat more subject to injury than that under normal bark. Some of the cambium within the lesion area is sometimes killed when none of that outside the lesion is destroyed. This takes place probably because new bark, developed in the sequence of the disease, is thinner in certain areas than normal bark. If the lesions are small, this result is not at all serious. It is also probable that use of two different strengths may be advisable, one for the trunk and larger limbs and the other for smaller limbs. In case of psorosis of citrus, however, it will not be advisable in any case to treat lesions on limbs much smaller than about 2 inches in diameter. Such limbs should be cut out instead of being treated.

SUMMARY

The results of these experiments indicate a promising chemical method for inducing bark-shelling with consequent production of new bark in psorosis lesions of orange trees. The advantages of such treatment over the bark-scraping method now in practice are obvious. This principle may have an application in the case of other bark diseases where the causal agent can thus be removed or the disease impeded by scraping or bark scarification. It is postulated that the best carrier for the toxic agent for such purpose will prove to be one that is only partially miscible with water and will, therefore, not too readily penetrate the bark and kill the cambium.

The two preparations so far tested that show the most promise in producing bark-shelling and at the same time do not seriously injure the cambium when applied with a brush to Valencia and Washington Navel orange bark, are dinitro-o-cyclohexylphenol (DNOCHP) at a concentration of approximately 1% by weight dissolved in kerosene and the same material dissolved in medicinal grade white mineral oil. A few tests indicate that ordinary gasoline without tetra-ethyl lead may also be a good carrier for the DNOCHP but in warm, dry weather, it evaporates too rapidly.

Lighter penetrants, carrying DNOCHP or other substances, are being tested.

BOTRYTIS LEAF SPOT OF VETCH[1]

J. L. WEIMER

(Accepted for publication, August 4, 1943)

INTRODUCTION

In March, 1940, Mr. George E. Ritchey, Gainesville, Florida, sent the writer some Augusta vetch (*Vicia angustifolia* L.) plants having numerous red spots on leaflets, petioles, and stems. These spots differed somewhat from those caused by any disease with which the writer was familiar or found described in the literature at the time. Although Augusta vetch grows wild in many parts of the South, it is used commercially only to a very limited extent, largely in Florida. Since this disease was evidently capable of doing considerable damage to Augusta vetch and possibly was potentially destructive to other vetch species of more commercial value, an investigation of its nature and cause has been made.

SYMPTOMATOLOGY

All aboveground parts, at least of young plants, are susceptible. On the leaflets the spots tend to be circular in outline but may be angular or irregular, especially when they are restricted by the larger veins. There may be only one spot on a leaflet or spots may be so numerous that most of the tissue of the leaflet is involved. These lesions are characteristically small, often from $\frac{1}{3}$ to $\frac{3}{4}$ mm. in diameter, and seldom over 1 mm. unless formed by the coalescence of two or more adjacent spots, or their growth is diverted by contact with veins. Their color may vary, but, usually, it is a shade of dark-red approaching oxblood of Ridgway.[2] The spot may be uniform in color or it may be ox-blood with a maroon border, or sometimes the border is claret-brown, mahogany-red, or chestnut, fading towards the center to light-brown, gray, or almost white. The spots may appear on either side of the leaflet and may or may not extend entirely through it. The affected areas are not excised but the leaflets are shed and a plant may be badly defoliated. On the stems and petioles the lesions are similar to those on the leaflets except they are commonly linear in shape, the longest axis being parallel to the corresponding axis of the part attacked. Such lesions are usually from $\frac{1}{2}$ to 1 mm. wide and up to 2 to 3 mm. long, but may enlarge, becoming 1 cm. or more long. The spots may continue to enlarge and coalesce with nearby lesions, so that girdling and death may result, small petioles and tendrils most commonly being killed in this manner. Damage to the plant results

[1] Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture and the Georgia Agricultural Experiment Station, Experiment, Georgia. Paper No. 117, Journal Series, Georgia Agricultural Experiment Station.

[2] Ridgway, Robert. Color Standards and Color Nomenclature. 43 pp., 53 color plates. (Washington, 1912.)

from the girdling of the parts affected, but perhaps more generally from defoliation. Considerable defoliation may result when the spots are numerous, as they often are under natural conditions (Fig. 1, A).

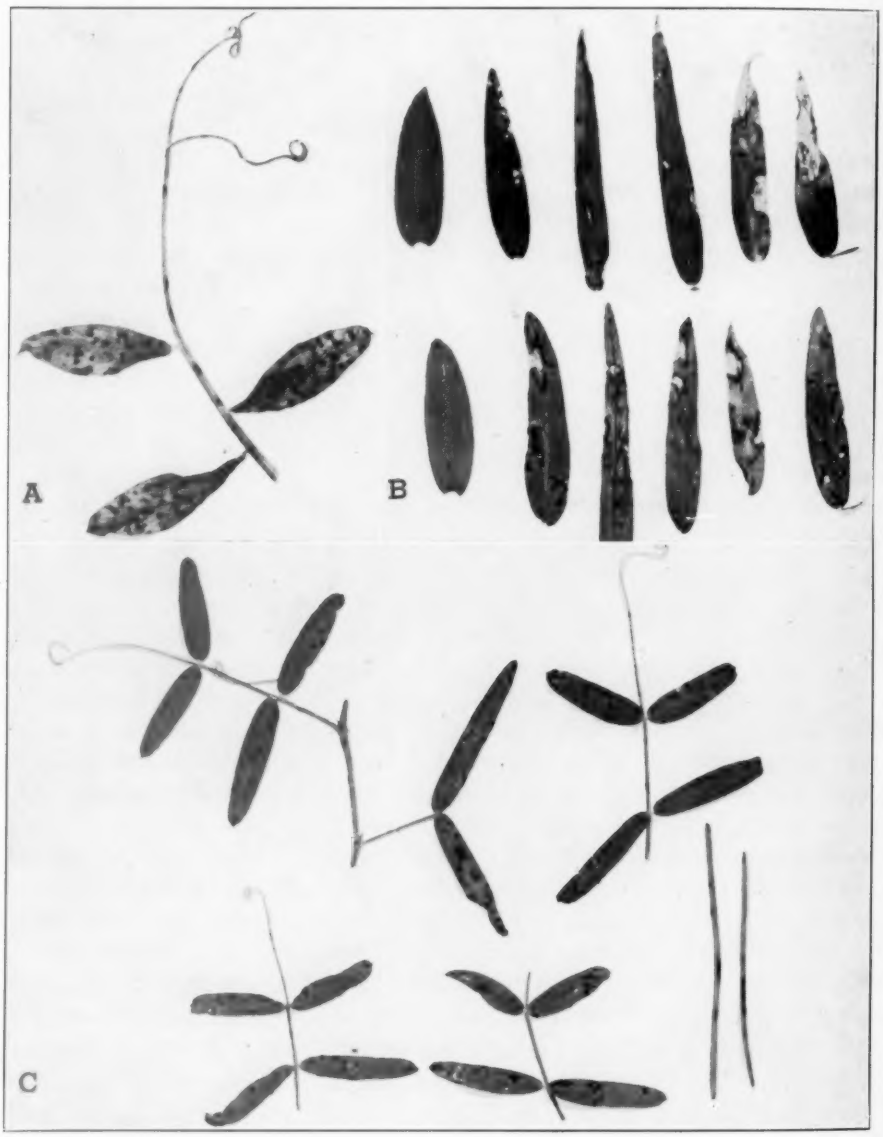

FIG. 1. *Botrytis* leaf spot of vetch. A. Naturally infected leaflets, leaf stalk, and tendril of *Vicia angustifolia* from Gainesville, Fla. ×6/7. B. Botrytis leaf spot on *V. grandiflora* resulting from inoculation. Two leaflets at the left are healthy. Upper and lower rows of leaflets show upper and lower surfaces, respectively. ×7/8. C. Botrytis lesions on leaflets, leaf stalks, stems, and tendrils of *V. sativa* (Alba) resulting from inoculation. About ×9/10.

ETIOLOGY

Pathogenicity

Isolations from the leaves received from Ritchey and from plants collected later by the writer showed the constant association of a *Botrytis* with these lesions. Inoculations demonstrated that the typical symptoms could be reproduced at will. On January 22, 1942, young plants of *Vicia angustifolia* grown in sterilized soil in the greenhouse were atomized with a suspension of spores from a young pure-line culture of the fungus growing on potato-dextrose agar and were then held in a moist chamber for 48 hours. Comparable control plants atomized with water only were placed in the moist chamber along with those inoculated. At the end of 48 hours, when the plants were removed from the moist chamber, there were numerous water-soaked, slightly sunken spots present on some of the leaflets of the inoculated plants but none on those of the controls. Four days later the spots had taken on the red color characteristic of the Botrytis disease. The fungus was recovered from several of the leaflets.

This experiment was repeated in much the same manner on November 24, 1942, and the disease again reproduced. The typical lesions were apparent 3 days after the inoculations were made. When lesions were very numerous, as along the margins or tips of the leaflets, the tissue was killed. The fungus was recovered from the lesions.

In order to ascertain whether or not other species of vetch also were susceptible to this disease, several species were grown in pots in the greenhouse and inoculated when the seedlings were about 6 to 10 inches tall. The following species were included in the experiment: *Vicia sativa* L. (F.C. 29933, F.C. 16462, F.C. 18808, Selection 7, Willamette, and Alba), *V. grandiflora* Scop., *V. atropurpurea* Desf., and *V. villosa* Roth. *V. angustifolia* was included as a known susceptible species. One pot of each lot, containing about 25 seedlings, was atomized with water as a noninoculated control and placed in a large moist chamber. Another pot of each lot was atomized with a suspension of the fungus spores and likewise placed in the moist chamber. The inoculations were made on January 6, 1943, and the pots were held in the moist chamber for 48 hours. The temperature of the chamber ranged from 18° to 20° C. during this period. When removed from the moist chamber none of the noninoculated controls showed any signs of disease, but lesions were evident on many of the inoculated plants. Infection was obtained on all of the lots tested with the exception of *V. villosa* and *V. atropurpurea*. The appearance of the lesions on the different species varied somewhat but were similar in most instances. The symptoms produced on *V. grandiflora* and *V. sativa* (Alba) are illustrated (Fig. 1, B and C). As shown in the figures, there was more tissue killed in *V. grandiflora* leaflets than in those of *V. sativa* (Alba) or of *V. angustifolia*. The lesions continued to enlarge somewhat after the plants were removed from the moist chamber, but seldom reached over 1 mm. in diameter. Considerable defoli-

ation took place in some lots, but, for the most part, as is often the case under natural conditions, the infection was not heavy.

TAXONOMY

The causal fungus is easily isolated and grows well on all media tried. It does not, however, sporulate well on any of them. Usually fruiting is most abundant at the top of long slants.

The mycelium is abundant, white to grayish in mass, varying from colorless to slightly grayish to brownish under the microscope, 4–11 μ in diameter and the branches often are constricted slightly where joining the parent hypha. The conidiophores are simple or branched and commonly elongate forming several fruiting heads. They are the same color as the mycelium, or slightly darker brown, the ends are enlarged, globoid or elongate, each bearing several spores on short sterigmata. Conidia are gray in mass, singly colorless or grayish to brownish like the mycelium, obovate, pyriform, or elliptical in shape and 13.7–25.2 × 8.4–15.8 μ (av. 18.4 × 11.6 μ) in size. Sclerotia were seen in culture only and were white at first, soon turning black, irregular in outline, often rounded when viewed from above but more or less hemispherical, and 1–4 mm. in largest diameter. The perfect stage was not seen.

There seems to be little doubt that this fungus belongs in the *Botrytis cinerea* group.[3]

DISCUSSION

After this work was nearly completed a reference to what is probably the same or a very similar disease was found in a paper by Wilson[4] on the chocolate spot of broad bean. During his investigations he tested the pathogenicity of the chocolate spot fungus on a number of legumes, among them being *Vicia sativa*. This species of vetch became severely infected as shown in his plate 17, figure 4. He does not describe the symptoms, however, hence it can only be assumed that they were identical with those of the same disease on *V. faba* L. If this be the case, presumably the color of the lesions was chocolate instead of the red characteristic of the disease of vetch studied by the writer. Wilson states further that in Great Britain vetch is reputed to suffer from chocolate spot, citing as authority the list of common names of British plant diseases.[5] The similarity between this disease of vetch and that of *V. faba* and the fact that Wilson found that several forms of *Botrytis cinerea* caused chocolate spot of *V. faba*, suggested the desirability of testing the pathogenicity of the vetch form of *B. cinerea* on *V. faba*. This was done by inoculating several plants with spores from a culture of the *Botrytis* from Augusta vetch. Four days after the inoculations were made red lesions, typical of the leaf spot on vetch, appeared on several *V. faba* leaflets from some of which the fungus was recovered. The lesions were slightly darker in color on *V. faba* than on the vetch species. The fact that this form of *B. cinerea* from vetch also attacked *V. faba* is of interest in view of the fact

[3] This conclusion was confirmed by Prof. H. H. Whetzel, Cornell University, Ithaca, N. Y., who kindly examined a culture of the fungus.

[4] Wilson, A. R. The chocolate spot disease of beans (*Vicia faba* L.) caused by *Botrytis cinerea* Pers. Ann. Appl. Biol. 24(2): 258–288. 1937.

[5] British Mycological Society. List of common names of British plant diseases. Trans. Brit. Mycol. Soc. 14: 140–177. 1929. (The disease at that time was attributed to *Bacillus Lathyri* Manns and Taubenh.)

that Wilson found that many forms of *B. cinerea* were capable of causing chocolate spot of *V. faba*.

Having learned that the form of *Botrytis cinerea* from vetch would attack *Vicia faba*, an attempt was made to determine whether the form of *Botrytis* from lupine[6] would also attack vetch. A single inoculation experi- All 4 strains of *V. sativa*, namely Willamette, Selection 7, Alba, and F.C. 18808, became infected. Purple vetch proved to be resistant, just as it was to the form of *Botrytis* from Augusta vetch. In this single test the form of *Botrytis* from lupine did not appear to be quite so pathogenic to vetch as did the form originally from vetch; although this may have no significance, since the environmental conditions and possibly other variable factors undoubtedly were not identical. The fact that all 3 of these hosts, vetch, lupine, and broad bean, are susceptible to these forms of *Botrytis* suggests that these crop plants should not be used in a rotation designed to control the Botrytis disease of any one of them.

Since the original collection of this disease on *Vicia angustifolia* was received from Gainesville, Florida, the same disease has been collected on this host and on *V. sativa* at Quincy, Florida, and also on *V. sativa* at Albany and Experiment, Georgia. Although the leaves were in some cases badly spotted the plants had made a good growth and the damage caused by the disease was slight.

CONTROL

Our knowledge of this disease indicates that for the present at least control measures are not necessary. Rotation should be helpful in preventing the rapid accumulation of inoculum in the field. Inoculation experiments indicate that Smooth and Purple vetches are highly resistant to the disease. *Vicia faba* and *Lupinus angustifolius* L. are susceptible, and, therefore, are not suitable for planting in a rotation designed to control this disease.

SUMMARY

A disease of vetch found in the Southeastern United States caused by a fungus of the *Botrytis cinerea* group is described. Small dark red spots are produced on the leaflets, stems, petioles, and tendrils of several species of the host. *Vicia villosa* and *V. atropurpurea* appeared to be resistant, while all strains of *V. sativa* tested, *V. angustifolia*, *V. grandiflora*, and *V. faba*, were susceptible. A form of *B. cinerea* from lupine also caused the leaf spot on vetch.

AGRICULTURAL EXPERIMENT STATION,
EXPERIMENT, GEORGIA.

[6] Weimer, J. L. A Botrytis disease of lupines. Phytopath. 33: 319–323. 1943.
ment was conducted, using the same methods as those described previously.

INVASION OF WATER-SOAKED TOBACCO LEAVES BY BACTERIA, SOLUTIONS, AND TOBACCO-MOSAIC VIRUS[1]

STEPHEN DIACHUN, W. D. VALLEAU, AND E. M. JOHNSON

(Accepted for publication August 23, 1943)

Previous reports (1, 3, 5) have shown that water-soaking of tobacco leaves enables leaf-spot bacteria (*Bacterium angulatum* and *Bact. tabacum*) to enter the leaves. Apparently, invasion occurs by way of water channels, presumed to exist between the outer leaf surface and the intercelluar spaces, through stomatal openings. It is not known whether the bacteria enter the leaf by virtue of their own active motility, *i.e.*, by "swimming," or by action of some other force. Knowledge concerning this point is of interest in studies on overwintering of the leaf-spot bacteria in soil (4) and on roots (7). In the method used to determine whether the bacteria are present in soil or on roots, a water suspension of the material to be tested is placed on water-soaked leaves of living plants. If infection occurs, it is concluded that the pathogenic bacteria are present. However, if infection does not occur, it may mean that pathogenic bacteria are not present at all, or that they are present but in an inactive or nonmotile state in which they may not be able to gain entrance. Thus, it is necessary to know whether active motility is essential for leaf invasion. The object of this report is to present indirect evidence indicating that motility is not necessary for bacterial entrance into leaves.

INVASION OF WATER-SOAKED TISSUE BY NONMOTILE BACTERIA

If nonmotile bacteria can invade water-soaked leaves as readily as motile organisms, the implication is that active motility is not necessary, and perhaps that any bacteria may be able to enter leaves without having to swim in. The following test was made to determine whether nonmotile bacteria can invade water-soaked tobacco leaves.

An area of a leaf was water-soaked by forcing a stream of water from a hypodermic syringe against the lower surface. A 24-hour beef-peptone-broth culture of *Staphylococcus aureus* diluted 1–10 with sterile water was poured on the lower surface of the water-soaked area. The area was marked, and, after water-soaking disappeared (30 minutes), was cut out, surface-sterilized 30 seconds in 1/1000 $HgCl_2$, and rinsed in 3 changes of sterile water. A disc was cut out with a sterile cork borer (7 mm. diameter), crushed in 10 cc. of melted potato-dextrose agar, and poured into a Petri plate. Five disc samples were thus tested. Within a few days thousands of colonies of *Staph. aureus* developed on each plate. In the same way, 5 disc samples were tested from a non-water-soaked area of the same leaf

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

inoculated with *Staph. aureus*. Not a single colony of the organism appeared on the plates.

Another area of the same leaf was water-soaked at the same time and inoculated with *Bacterium angulatum*. Sample discs were tested in the same way. Thousands of colonies developed on each plate prepared from tissue water-soaked when inoculated, while no bacteria were recovered from a non-water-soaked inoculated area. This test was repeated 3 times with similar results (Table 1).

The cells of *Staphylococcus aureus* are believed to be nonmotile, while those of *Bacterium angulatum* are motile rods. This test shows that a motile organism has no advantage over a nonmotile organism in entering water-soaked leaf tissue. It does not prove that *Bact. angulatum* enters without swimming, but suggests that it can do so.

TABLE 1.—*Recovery of motile and nonmotile bacteria (Bacterium angulatum and Staphylococcus aureus) from within tobacco leaves inoculated by pouring bacterial suspension on water-soaked tissue*

Organism	Leaf treatment	Number samples tested	Approximate number of colonies on agar from within crushed leaf samples 38.5 mm.² in area		
			Test 1[a]	Test 2	Test 3
Staph. aureus	Water-soaked	5	5,000 to 20,000	1,000 to 3,000	20 to 100
" "	Non-water-soaked	5	0	0	0
B. angulatum	Water-soaked	5	2,000 to 10,000	2,000 to 5,000	150 to 800
" "	Non-water-soaked	5	0	0	0

[a] In Test 1 the 24-hour broth culture used as inoculum was diluted 1 to 10 with water; in Test 2 the dilution was 1 to 100; in Test 3 the dilution was 1 to 1000.

ENTRANCE OF INDIA INK INTO WATER-SOAKED LEAVES

To determine whether nonliving particles can enter water-soaked leaf tissue, India ink was placed on water-soaked areas and adjacent non-water-soaked areas of the same leaf. Invariably the ink entered only the water-soaked areas, producing a blackening of the leaf, visible through both upper and lower surface, that could not be washed off. Entrance apparently was instantaneous, for, even when the ink was washed off immediately after being put on, blackening occurred. Examination of strips of lower epidermis showed accumulation of ink in the stomata. Free-hand sections of leaves showed ink to be confined principally to the surface of spongy cells. The ink in this condition apparently is not toxic to the cells.

ENTRANCE OF SOLUTIONS INTO WATER-SOAKED LEAVES

To determine whether solutions of toxic chemicals can enter water-soaked leaves, the following test was made. The left side of 3 leaves was water-

soaked. A 1–1000 solution of $HgCl_2$ was poured onto the lower surface of both left and right sides, and the leaf was then rinsed with tap water. As the water-soaking disappeared, the tissues collapsed and soon became dry. In spots the entire thickness of the leaf was reduced to a thin parchment-like membrane; other regions of the water-soaked areas collapsed only on the lower surface, which then appeared rough and gray, while the upper surface remained normal. The degree of injury seemed to depend on whether the chemical injured the spongy cells only, or both spongy and palisade cells. This experiment was repeated several times with similar results. Similar injury was produced by $CuSO_4$ (1–100) and Bordeaux mixture (3–3–50) applied to water-soaked tissues. Injury in each case was confined to the water-soaked areas.

ENTRANCE OF TOBACCO MOSAIC VIRUS INTO WATER-SOAKED LEAVES

Caldwell (2) has injected leaves of *Nicotiana glutinosa* with tomato aucuba-mosaic virus without producing infection. Johnson (6) has shown

TABLE 2.—*Recovery of tobacco mosaic virus from within tobacco leaves inoculated by placing infectious leaf extract on water-soaked tissue*

Test	Replication	Number of necrotic spots produced on leaf of NN burley plant		
		Rubbed with surface-sterile tissue from		Water-soaked when in contact with virus, without further treatment
		Leaf water-soaked when in contact with virus	Leaf not water-soaked when in contact with virus	
1	1	51	9	0
	2	8	0	0
2	1	54	0	0
	2	58	0	0
3	1	49	0	0
	2	35	0	0

that water-soaked leaves of an *N. glutinosa* × *N. tabacum* hybrid do not become infected when sprayed with tobacco-mosaic virus. The following tests show that tobacco-mosaic virus placed on the surface of water-soaked leaves enters the leaves but does not cause infection.

An interveinal area of a leaf of a *Nicotiana digluta* × *N. tabacum* hybrid containing the N factor was water-soaked, and immediately leaf extract from a mottled mosaic-diseased burley plant was placed on the lower surface of this area. After the water-soaked condition disappeared (30 minutes), the area was tested to determine whether virus was present inside the leaf. The area was cut out, washed in concentrated trisodium phosphate 60 seconds to destroy virus on the outer surface, rinsed in running tap water, crushed, and rubbed on another leaf of the same plant. Three days later numerous necrotic spots were present on the rubbed area. A non-water-soaked area, on which infectious juice was placed at the same time, was cut out, washed in trisodium phosphate, rinsed, crushed, and rubbed onto

a leaf. It produced no necrotic spots, showing that virus particles were not carried on the leaf surface.

Infectious juice was placed on a similar water-soaked area without further treatment. Infection did not occur. These tests show that tobacco-mosaic virus can enter leaves through stomata of water-soaked areas without producing infection.

Each test was performed in duplicate, and repeated 3 times (Table 2).

CONCLUSION

The tests reported in this paper show that nonmotile bacteria, as well as virus, non-living particles (India ink), and solutions, can enter water-soaked leaves. Pathogenic leaf-spot bacteria need not be motile or free-swimming to invade leaves.

The fact that chemicals can enter water-soaked tissue and cause injury suggests the possibility that naturally induced water-soaking may play a part in the occurrence of spray injury.

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PHYTOPATHOLOGICAL NOTES

Use of Liquid Culture of Fusarium for Field Inoculation of Cotton.—Field testing of cotton breeding material for resistance to *Fusarium vasinfectum* has been handicapped by difficulty in obtaining uniform infection. Attempts to build up natural infestation in field soils by the addition of artificial cultures, on solid media such as oats, corn meal, etc., and infected cotton stalks have been laborious, cumbersome and not entirely successful.

From infection results in greenhouse tests obtained with liquid inoculum with the blackroot-rot organism by Allison and with Fusarium wilt of cotton by Tharp and by Sherbakoff, it seemed likely that liquid inoculum also might be employed successfully in the field. In the method employed by the senior writer in greenhouse tests at Knoxville, the liquid inoculum was introduced easily and effectively through deep holes in the sand, close to the plant roots. The following adaptation of the method was employed in the field at Knoxville in 1942.

The inoculum was prepared as follows: The fungus was cultured on thin layers of agar in standard Petri plates. Several days later small circular discs of about 3 mm. diameter of the growth were cut out and placed on the wet inside surface of a 500-cc. Erlenmeyer flask containing 300 cc. of nutrient solution,[1] spore side to the glass. This was for the purpose of washing out most of the spores into the liquid, and afterwards the discs were washed down into the flasks. For a two-day period the inoculated flasks were thoroughly shaken every 3 or 4 hours during the day time, and were then used for inoculating an unsterilized solution composed of 1 part of table syrup to 100 parts of water at the rate of 1 flask (300 cc.) to 3 gal. of the sweetened water. The bulk culture was prepared in a barrel. During the preparation of the liquid cultures in the flasks and in the barrel a temperature of about 80° F. was maintained. The culture in the barrel was stirred frequently and at the end of 48 hours was ready for use.

The cotton under test was planted in the field June 22, in hills of 5 seeds each, around a wooden peg $1'' \times 1'' \times 10''$, set about 4″ deep in the ground. On July 15, when the plants were about 2 weeks old, the pegs were removed and one-half pint of the inoculum was poured into the hole. The hole was then refilled with soil. The soil is classified as Decatur silt loam, usually considered unfavorable for the development of wilt.

Observations of the amount of wilt were made at frequent intervals. At the end of the growing season the amount of internal discoloration was determined by cutting the stalks. Results of field inoculation of 23 varieties or strains of cotton are given in table 1. Among these varieties wilt infection varied from 0 to more than 50 per cent. The behavior of the check varieties,

[1] Each liter of nutrient solution contained:
10 grams—Sugar
5 grams—Magnesium sulphate
1 gram —Potassium phosphate
1 gram —Ammonium nitrate

TABLE 1.—*Results of field inoculation of cotton varieties with Fusarium vasinfectum, Knoxville, Tennessee, 1942*

Variety and pedigree	Hills	Plants			Plants with wilt symptoms		
		Total	Healthy	Diseased	Dead or defoliated	Internally discolored	Total
	Number	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
Delfos 719 (339)	15	56	40	16	23.2	5.4	28.6
Delfos 719 (992)	15	44	37	7	13.6	2.3	15.9
Acala 911 (330–1–8–2)	15	52	33	19	30.8	5.8	36.6
Acala 911 (330–1–8–4)	15	54	32	22	33.3	7.4	40.7
Trice (2–6)	15	53	37	16	20.7	9.4	30.2
Cook 307 (Resistant check)	12	19	19	0	0.0	0.0	0.0
Acala 911 (330–1–1–8)	15	54	26	28	38.9	13.0	51.9
Coker 33–12 (289–1)	15	48	38	10	20.8	0.0	20.8
Coker 33–12 (289–3)	15	50	24	25	44.0	6.0	50.0
Coker Wilds (176)	15	45	24	19	37.8	4.4	41.2
Delfos 9252 (182)	15	53	34	14	18.9	7.5	25.4
D.P.L. 11A (259)	15	38	28	9	15.8	7.9	22.7
Stoneville 5 (62)	15	57	32	25	38.6	5.3	43.9
Coker 100 (994)	15	45	25	20	31.1	13.3	44.4
Coker Wilt 100 (Resistant ck.)	15	48	43	4	4.2	4.2	8.3
Seabrook #10 (S.I.) (Res. ck.)	14	25	24	0	0.0	0.0	0.0
D.P.L. 11 (51)	15	51	33	18	27.5	7.8	35.3
D.P.L. 4–8 (50)	14	35	33	2	0.0	5.7	5.7
Stoneville (37–13)	15	50	43	6	8.0	4.0	12.0
Trice × Tidewater (578)	14	40	19	21	42.5	10.0	52.5
Stoneville (17–5–8)	15	51	35	15	21.6	7.8	29.4
Stoneville 37 × Stoneville 5	15	50	42	7	6.0	8.0	14.0
Half and Half (Susceptible ck.)	12	24	14	10	25.0	16.7	41.7

Cook 307, Coker Wilt 100, Seabrook #10 (Sea Island), and Half and Half, closely approximates the expected response, based upon previous inoculation trials in the greenhouse and on common field experience.

Although experience with this method has been limited, it does seem to have real promise for field tests. The cost of the culture medium is negligible, and very little time is required in preparation of the inoculum and in making the field inoculations. Results indicate that the method gives a reliable measure of the resistance of the cottons to fusarium wilt.—C. D. SHERBAKOFF, PAUL R. MILLER and D. M. SIMPSON, Tennessee Agricultural Experiment Station, Knoxville, Tenn.

Nicotine Fumigation Injury in Biloxi Soybean.[1]—The leaves of Biloxi soybean plants, which were used in a series of photoperiodic studies over a

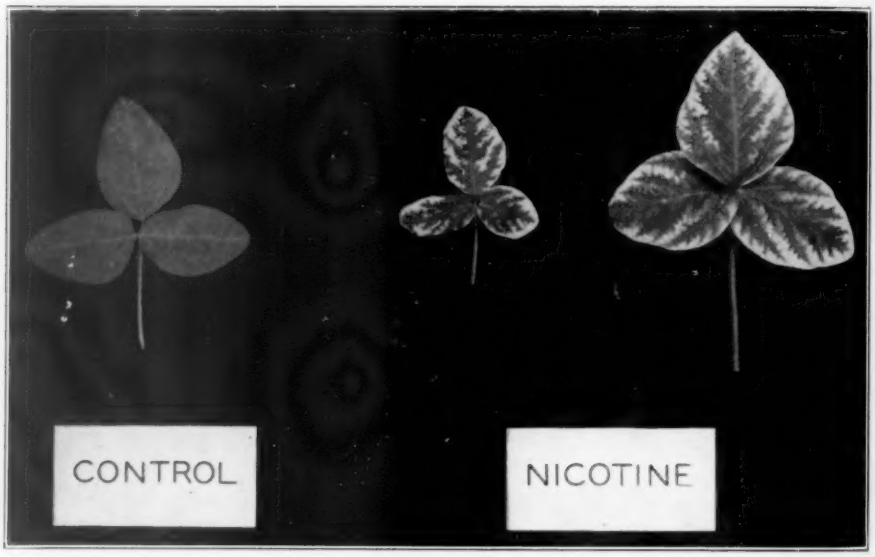

FIG. 1. Nicotine fumigation (right) causes severe and permanent chlorosis in the leaves of Biloxi soybean.

period of several years, frequently exhibited a severe permanent chlorosis, which appeared at the leaf margins and interveinally, occurring at all seasons of the year. Occasionally the injury was so severe that it was necessary to discard the plant material. The plants were grown in a greenhouse in subirrigation nutrient-solution culture, and it was thought at first that a nutritional unbalance or that unfavorable temperature conditions might be responsible for the symptoms. However, variations in nutrition and variations over a wide range of air and root temperatures failed either to produce the symptoms or to cause them to disappear. Finally, it was noticed that the chlorosis invariably followed nicotine fumigation. Therefore, the following investigation was made.

[1] Journal Paper No. 121 of the Purdue University Agricultural Experiment Station.

Test plantings were made in each of two greenhouse units. In one unit, the house was fumigated with a pressure fumigator, using a nicotine fumigant, containing not less than 14 per cent of nicotine, at the rate of one pound per 20,000 cubic feet of air space. Care was taken to make certain that the fumigant was not ejected directly on the plants. The ventilators were opened at the end of 3 hours, but the fumigation was done with outside-air temperature above freezing so that most of the fumigant escaped through the imperfectly sealed glass before the end of the fumigation period. No fumigation was done in the second greenhouse unit.

Within 24 hours after the treatment, the fumigated plants began to show marginal chlorosis on the young unfolding leaves and in about 4 days the typical marginal and interveinal chlorosis was manifest (Fig. 1). The photograph was taken 30 days after fumigation. The rapidity of development of the symptoms depended on the rate of growth. Where rapid growth occurred, the symptoms appeared more quickly. The injury was confined largely to the young, developing leaves. Those within the bud showed little injury. If the older leaves were damaged, the chlorosis usually was not so severe in them and the injured leaf tissue became brown and necrotic, usually in 10 days to 2 weeks. Subsequent fumigations made without the use of the pressure fumigator have resulted in injury similar to that described above.

While the writers have assumed that the injury is due to nicotine, it is entirely possible that it was caused by products derived from combustion accelerators or to other products of the fumigant.—ALICE P. WITHROW and J. P. BIEBEL, Department of Horticulture, Purdue University, West Lafayette, Indiana.

Cercospora Fruit and Leaf Spot of Olive.—In May, 1941, a grower in Solano County, California, called our attention to olive fruits that had remained on the trees during the winter without normal blackening over the entire surface. The stem half of the fruits had remained green, except for scattered purple spots. Stained radial sections through these spots revealed that the tissues beneath were heavily infected by very small hyphae that grew between the host cells, but did not enter the cells and did not kill them.

In November, 1941, W. T. Horne and I. J. Condit sent us olive fruits from Southern California that showed similar purple spots on green fruits (Fig. 1, A). Sections and cultures from these fruits revealed the same fungus observed in the olives from Solano County.

The fungus is very slow-growing in culture (Fig. 1, B) and may, therefore, not be detected by the usual isolation technique. After the fungus has been in culture for some time it produces small pycnidiumlike structures (Fig. 1, C, a) containing minute unicellular spores, which we have not been able to germinate. It appears probable that these spores may be spermatia (Fig. 1, C, b). Multiseptate conidia are later produced in culture.

Infections on leaves may not be detected unless a careful search is made. They result in the formation of indistinct darkened areas on the lower sur-

face of the leaves and may cause early drop of infected leaves. The imperfect fructifications of this fungus have been observed on the discolored spots on overripe fruits (Fig. 1, D) and growing from stomata on the lower surface of leaves. The spores are similar to the multiseptate conidia produced in agar cultures, but are slightly shorter when growing on the host.

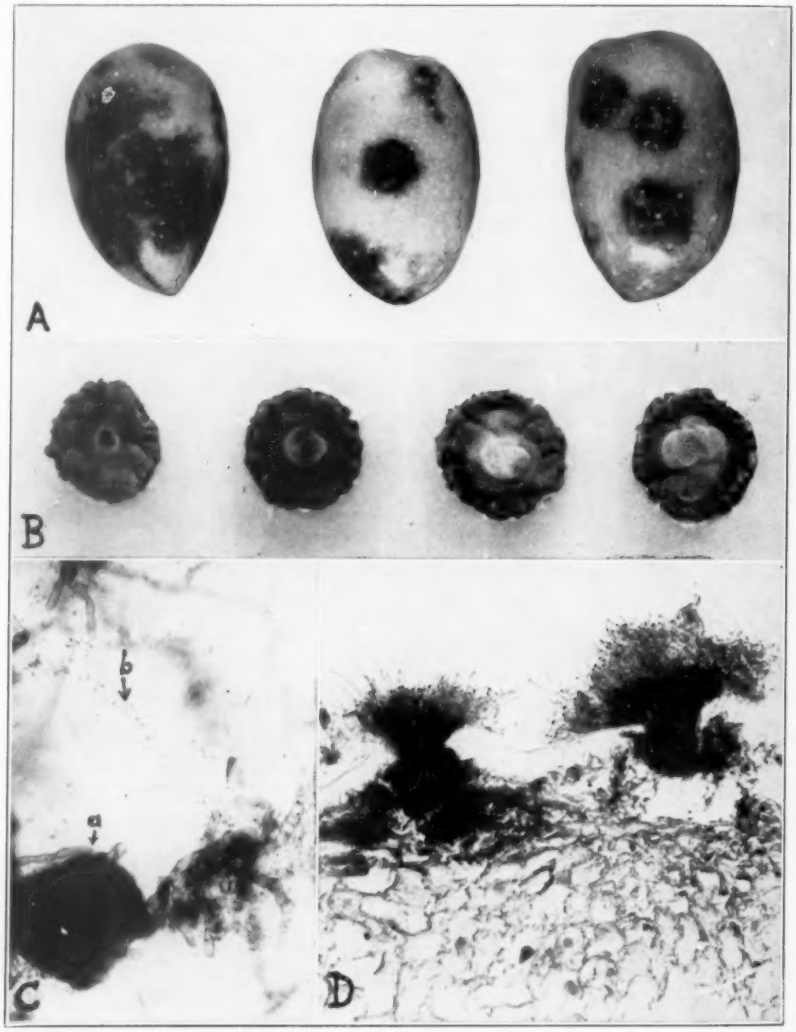

FIG. 1. A. Symptoms of Cercospora infections on olive fruits. B. Fourteen-day-old colonies of fungus on potato-dextrose agar. C. Spermogonium (a) and spermatia (b). D. Section through overripe olive fruit showing conidial tufts breaking through the cuticle.

The size and septation of the spores are similar to those of *Cercospora cladosporioides* Sacc. reported by Saccardo on olive leaves in Italy and Algeria. It, therefore, appears probable that we are dealing with the same species described by Saccardo.

In California this fungus has caused significant damage to fruits that are to be pickled as green olives. R. H. Vaughn of the Fruit Products Division, University of California, has found that infected fruits show the discolored spots after pickling and must be discarded. However, infected fruits are satisfactory for pickling as ripe olives. After the ripe-olive pickling process the infected, as well as the uninfected, portions of the fruits assume the same desired dark color. The flavor and texture of the fruits are apparently not impaired by the fungus, and no evidence of toxicity has been noted by individuals who have eaten large quantities of the ripe pickled fruit.—H. N. HANSEN and T. E. RAWLINS, Division of Plant Pathology, University of California, Berkeley, California.

New Strain of Agrobacterium rubi from Boysenberry.—The first serious outbreak of cane gall on boysenberry, *Rubus ursinus* var. *loganobaccus*,[1] thus far noted by the writer occurred in a planting near Auburn, New York, in the spring of 1942. The cultures obtained from these galls (Fig. 1, A) were studied bacteriologically and appeared identical in growth character, morphology, and physiology to those previously reported by the writer[2] and to those isolated about the same time as the above from black raspberry, *R. occidentalis* L., collected in a local garden.

Pathogenicity tests with cultures from both sources made into new black raspberry canes in midsummer all behaved similarly in inducing galls at the points of inoculation. When the galls thus induced on the new canes were removed by pruning the canes at variable distances below the position of the galls in late October, new galls appeared on the majority of canes the following season. These galls occurred in cases where the pruning cuts had been made as much as 8 to 12 inches below the gall. This is positive evidence that the cane-gall organism was able to migrate through first-year canes late in the season as they approach dormancy.

In making a comparative study with two other cell-stimulating organisms, *Agrobacterium tumefaciens*[3] and *A. rhizogenes*, on Bonny Best tomato, Cumberland black raspberry, and *Kalanchoe daigremontiana*, it was discovered that on wound inoculation the boysenberry strains, but not those from black raspberry, stimulated tiny galls (2 mm.) on *Kalanchoe* (Fig. 1, B) within 1 month. Reisolations demonstrated the presence of a pathogen identical to the original in the tiny galls. Excision of the tiny galls resulted in the reappearance of galls at the original sites of inoculation

[1] BAILEY, L. H. Species Batorum. The genus Rubus in North America. Gentes Herbarum. 5(1): 1–64. 1941.

[2] HILDEBRAND, E. M. Cane gall of brambles caused by *Phytomonas rubi* n. sp. Jour. Agr. Res. [U.S.] **61**: 685–696. 1940.

[3] The taxonomy of the cell-stimulating pathogens, of which *Phytomonas tumefaciens* (Sm. and Town.) Bergey *et al.* is the type species, has been recently changed by Conn (Conn, H. J. Validity of the genus Alcaligenes. Jour. Bact. **44**: 353–360. 1942) to the new genus *Agrobacterium*. This designation for the cane gall organism, *Agrobacterium rubi*, was first used by Starr and Weiss (Starr, M., and J. E. Weiss. Growth of phytopathogenic bacteria in a synthetic asparagin medium. Phytopath. **33**: 314–318. 1943.

(Fig. 1, B). Reinoculations into *Kalanchoe* again resulted in tiny galls. Because only the boysenberry strains of the cane gall organism were capable

FIG. 1. A. Symptoms of cane gall, occurring naturally on boysenberry and completely involving a section of stem about 1 foot long. $\times \frac{1}{2}$. B. Tiny galls induced on Kalanchoe by a boysenberry strain of cane gall organism 5 months after inoculation. The two lower galls were excised a little over a month before the photograph was made and were replaced by the galls shown. $\times \frac{3}{4}$.

of inducing tiny galls on *Kalanchoe*, they apparently represent a new pathogenetic strain, which extends the host range of this organism beyond the genus *Rubus*.—E. N. HILDEBRAND, Cornell University, Ithaca, N. Y.

BOOK REVIEW

DODGE, BERNARD O., and HAROLD W. RICKETT. *Diseases and Pests of Ornamental Plants.* xi + 638 p. illus. (figures not serially numbered). The Jaques Cattell Press, Lancaster, Pa. 1943. $6.50.

Although several experiment station bulletins dealing in general with the diseases and pests of ornamental plants have been issued in the United States in recent years, and although limited parts of this field, especially that of ornamental trees and shrubs, have been covered in at least two commendable manuals, the book under review is the first to attempt the whole range of ornamentals and to cover all manner of ailments and pests. Since the subject matter of the ornamental plant field is so heterogeneous—some one somewhere probably has regarded almost every kind of plant that grows as having decorative value—the task of compiling the relevant disease and pest information is truly formidable. When to this difficulty is added that of an extreme disparity in the value attaching to the currency that circulates in this realm—some of it sound and much of it otherwise, because floriculturally-minded persons, although voluminous writers, have not been until recently very scientific—it follows that standards of thoroughness and accuracy to be expected in books dealing with the pathology of economic crops cannot yet be demanded here.

This book may be appraised for (1) its value as a handbook for gardeners whether amateur or commercial, and (2) as a reference book for professional plant pathologists (including entomologists!). For amateur gardeners it brings together in convenient form a large amount of information that is otherwise inaccessible or is widely scattered in bulletins, periodicals, and other books. The subject of disease and pest control is presented with thoroughness and on the whole with up-to-date information, reflecting the senior author's experience with a wide variety of plants under garden and greenhouse conditions. Somewhat less familiarity is indicated with the pathological problems of commercial culture of certain ornamentals, flowering bulbs for example, in the statements, p. 424 *et seq.* (1) that basal rot is not serious on the trumpet type of narcissus, whereas it is by far the most important disease of commercial narcissus culture, consisting largely of trumpet types, in this country; (2) in the recommendations of long soaking treatments for the control of basal rot, whereas commercial growers have found only short dip treatments (which are not mentioned at all) to be practicable; (3) the vector of narcissus mosaic is not known, whereas a number of aphid species have been shown to transmit this virus. Other cases in point are omission of the highly injurious pests of *Gerbera*, the cyclamen and bulb mites under greenhouse culture, and root knot in both greenhouse and nursery plantings; in omission of all reference to the Heteroceras spot of cultivated *Nymphaea;* the statement that *Sclerotium rolfsii* is not reported on tulips in America, whereas this fungus, or its form *S. delphinii*, causes an important field disease of commercial tulip culture in both Long Island and North Carolina.

Professional workers in this field will find the assemblage of pathological and entomological information very useful, although there are some important omissions, and other items are included apparently on the sole basis of their mention in European literature without reference to their occurrence or importance here.—FREEMAN WEISS, U. S. Bureau of Plant Industry Station, Beltsville, Maryland.

FUNGI FOR PENICILLIN PRODUCTION

A project is being organized at the University of Minnesota Agricultural Experiment Station, Division of Plant Pathology on Botany, to survey Penicillia belonging to the *Penicillium notatum* group and also species of *Aspergillus* for the production of penicillin. The project is under the supervision of Dr. E. C. Stakeman. Cultures of organisms are desired, and individuals are requested to forward isolations of the groups of fungi mentioned to the laboratory indicated. Isolations known to produce penicillin are especially desired.

Individuals who wish to survey other fungi for penicillin activity can obtain directions for a standard technique from the United States Department Agriculture, Regional Laboratory at Peoria, Illinois.

ALBERT L. ELDER
War Production Board
Coordinator of Penicillin Program